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Asynchronous pulse responses of soil carbon and nitrogen mineralization to rewetting events at a short-term: Regulation by microbes

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Rewetting after precipitation events plays an important role in regulating soil carbon (C) and nitrogen (N) turnover processes in arid and semiarid ecosystems. Here, we conducted a 48-h rewetting simulation experiment with measurements of soil C and N mineralization rates (R_C and R_N , respectively) and microbial biomass N (MBN) at high temporal resolution to explore the pulse responses of R_C and R_N . R_C and R_N responded strongly and rapidly to rewetting over the short term. The maximum R_C value (because of pulse effects) ranged from 16.53 to 19.33 $\mu\text{g C g}_{\text{soil}}^{-1} \text{h}^{-1}$, observed 10 min after rewetting. The maximum R_N varied from 22.86 to 40.87 $\mu\text{g N g}_{\text{soil}}^{-1} \text{h}^{-1}$, appearing 5–6 h after rewetting. The responses of soil microbial growth to rewetting were rapid, and the maximum MBN was observed 2–3 h after rewetting. Unexpectedly, there was no correlation between R_C , R_N , and MBN during the process of rewetting, and R_C and R_N were uncoupled. In sum, the pulse responses of R_C , R_N , and microbial growth to simulated rewetting were rapid, strong, and asynchronous, which offers insights into the different responses of microbes to rewetting and mechanisms behind microbes.

Periodic rewetting of soil after precipitation events is common and exerts a significant influence on the carbon (C) and nitrogen (N) cycles of an ecosystem^{1–3}. Precipitation events are the main drivers of the N cycle in soil and plant growth in arid and semiarid areas, which all depend on precipitation intensity and previous soil water conditions^{2–4}. Birch H.F⁵ first reported that precipitation events after long-term or severe drought lead to strong and rapid increases in soil respiration and microbial biomass through a pulse or priming effect. Huxman, *et al.*¹ reviewed the pulse effects of precipitation events on plants, soil, and physiological ecosystem activity and found significant impacts on the C and N cycles in soil and productivity in arid and semiarid ecosystems where small precipitation events can trigger these pulse effects. Borken and Matzner⁶ reappraised the effects drying-wetting effects on soil C and N mineralization and fluxes, and found that increasing summer droughts may reduce the mineralization and fluxes of C and N whereas increasing summer precipitation can enhance the losses of C and N from soils. Global climate models predicted that the frequency and intensity of drying-rewetting events are likely to alter due to future climatic change⁷. These future scenarios highlight the importance of understanding how drying-rewetting cycles will influence dynamic of soil C and N cycles^{7,8}. For example, a recent meta-analysis study that investigated drought effects on soil C cycles and found that greater C were loss from C-rich (>2% organic C) soil during whole drying-rewetting cycles⁸.

Previous studies have explored different aspects of pulse effects in arid and semiarid areas^{9,10}. Birch⁹ found that those soils subjected to short-term drought may experience greater C and N mineralization rates after a

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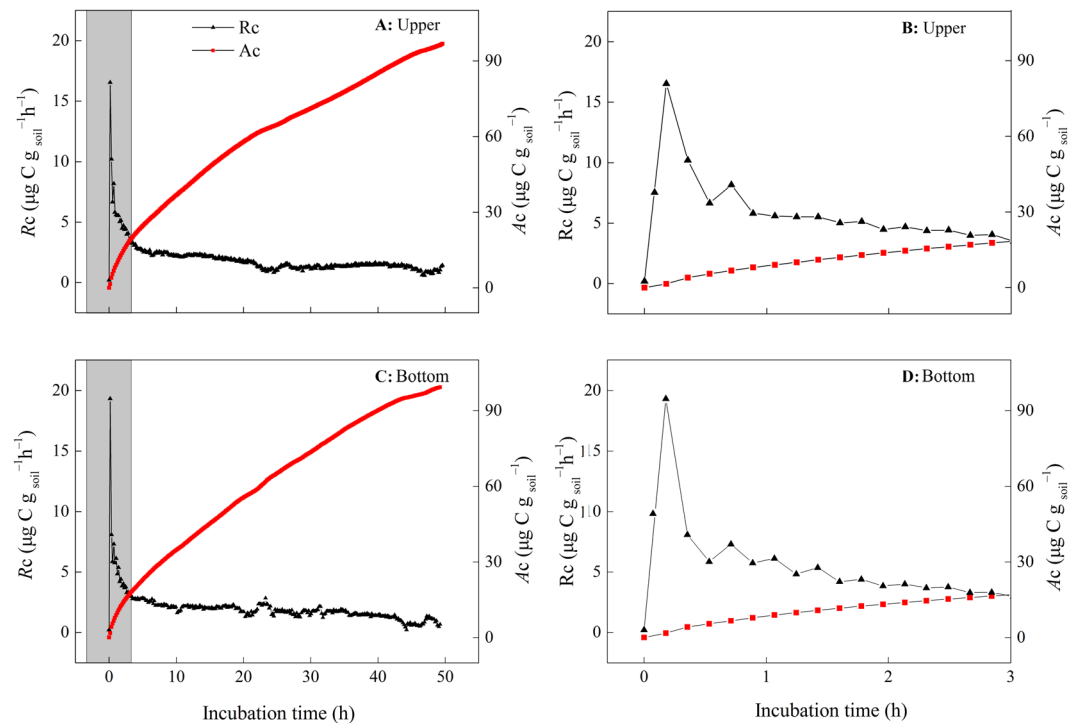


Figure 1. Dynamics of soil carbon mineralization rates (R_C , $\mu\text{g C g}_{\text{soil}}^{-1} \text{h}^{-1}$) and accumulation (A_C , $\mu\text{g C g}_{\text{soil}}^{-1}$) through simulated rewetting. Panels B and D were the amplification of shades in panels A and C, respectively. Upper and Bottom represented the sampling locations along a slope.

short period of rewetting than these soils with constant soil water content. Sponseller¹⁰ investigated the rapid pulse responses of microbes and soil respiration to short-term precipitation in the Sonoran Desert at intervals of 15 and 30 min and 1, 2, 5, 24, and 48 h. Dijkstra *et al.*¹¹ found that microbial fixation, total soil N mineralization, and nitrification rate increased rapidly within one to three days. As proposed by Huxman, *et al.*¹, the pulse effects of precipitation events are time-sensitive and may be classified as short-term sharp rise (minute or hour) or long-term stable increase (day or month). Jones and Murphy¹² found that the response of soil microbes to added artificial substrates (sugars and amino acids) and rainwater separately was instantaneous, peaking in less than 16 min, which suggested that the rapid responses of soil C and N mineralization to rewetting are rapid and strong at initial stage. To date, however, no study has focused on the short-term pulse effects of precipitation events or rewetting on both soil C and N turnover from minutes to several days.

Soil C and N mineralization, driven by microbial activity, are often closely linked without the limitations of soil water content^{13–15}. In the process of rewetting, microorganisms play two important roles as regulators of soil C and N mineralization and reservoirs of soil nutrients^{1, 16}. Through the pulse effects of rewetting, microbial biomass and activity in soil first increase sharply, rapidly decomposing soil organic matter (SOM) to satisfy microbial requirements for energy and elements (C, N, and P) within a very short time. The disproportional C:N ratios of SOM and soil microbes result in a sharp increase in soil CO_2 emissions due to the relative stability in C:N ratios required by soil microbes^{17, 18}. After rapid microbial growth, soil N mineralization rates are expected to increase as a result of an increase in microbe metabolism and microbe death accompanying with the depletion of labile SOM⁵. Finally, the C:N ratios arrive at the relatively stable status required by microbes, and the often coupled ratios of soil C and N mineralization may be observed, as in previous studies^{13, 19}. We therefore assumed that the different roles of soil microbes under different phases of rewetting may result in uncoupled C and N mineralization at a short-term. Unfortunately, few studies have explored the coupled relationship between soil C and N mineralization during the initial phase of rewetting event^{1, 9–11}.

Based on a 48-h incubation experiment of soil C mineralization rate (R_C , 6-min intervals), N mineralization rate (R_N , 18 times), and microbial biomass nitrogen (MBN, 18 times) at high temporal resolution, we investigated the dynamics of R_C , R_N , and MBN in two semi-arid forest soils through a simulation of rewetting. The main objectives of this study were (1) to investigate the short-term dynamics of R_C , R_N , and MBN after rewetting; (2) to quantify the pulse effects of simulated rewetting on R_C and R_N ; and (3) to discuss the underlying mechanisms influencing R_C and R_N in initial phase of rewetting event. We specifically tested the hypothesis that the short-term responses of soil microbes to rewetting may drive different processes of and an uncoupled relationship between R_C and R_N .

Results

Dynamics of R_C and pulse effects of rewetting. R_C increased immediately and sharply, peaking after 10–20 min of simulated rewetting (Fig. 1), and then apparently decreased with prolonged incubation, irrespective

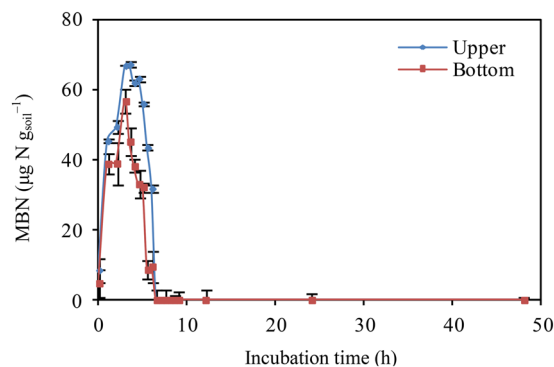


Figure 2. Dynamics of soil microbial biomass nitrogen (MBN, $\mu\text{g N g}_{\text{soil}}^{-1}$) after rewetting.

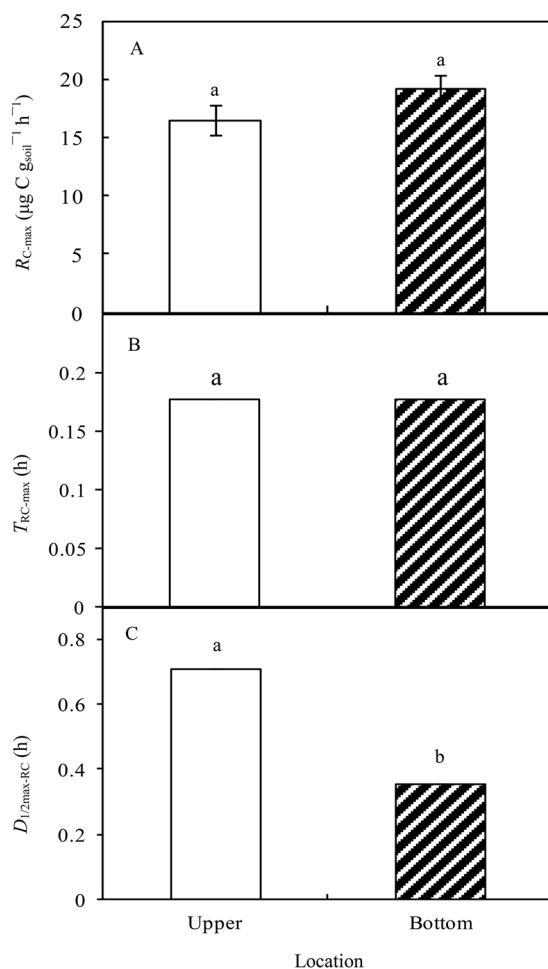


Figure 3. Pulse responses of soil carbon mineralization to simulated rewetting: (A) maximum soil carbon mineralization rate ($R_{C-\text{max}}$, $\mu\text{g C g}_{\text{soil}}^{-1} \text{h}^{-1}$), (B) time to reach $R_{C-\text{max}}$ ($T_{RC-\text{max}}$, h), (C) duration from $1/2 R_{C-\text{max}}$ to $1/2 R_{C-\text{max}}$ ($D_{1/2\text{max-RC}}$, h) being used to express the duration of pulse effect. Data with same superscript letters within each column indicate no significant difference at $P = 0.05$.

of location (upper or bottom). Similar trends were observed with MBN, although a time lag was apparent, with a peak about 3–4 h after rewetting (Fig. 2). No significant relationships between R_C and MBN were observed from the early stages through the peaking of R_C during the 48-h incubation period.

Rewetting exerted strong pulse effects on R_C . The values of $R_{C-\text{max}}$ were 16.53 and 19.33 $\mu\text{g C g}_{\text{soil}}^{-1} \text{h}^{-1}$ for the upper and bottom samples, respectively, with no significant difference between the locations (Fig. 3A). $T_{RC-\text{max}}$ was observed at 0.18 h in both locations (Fig. 3B). $D_{1/2\text{max-RC}}$ was significantly higher in the upper (0.71 h) than the bottom location (0.36 h) ($P < 0.05$, Fig. 3C). A_C was 109.34 $\mu\text{g C g}_{\text{soil}}^{-1}$ in the upper location and 114.35 $\mu\text{g C g}_{\text{soil}}^{-1}$ in the bottom location, showing no significant difference between them (Figures not shown).

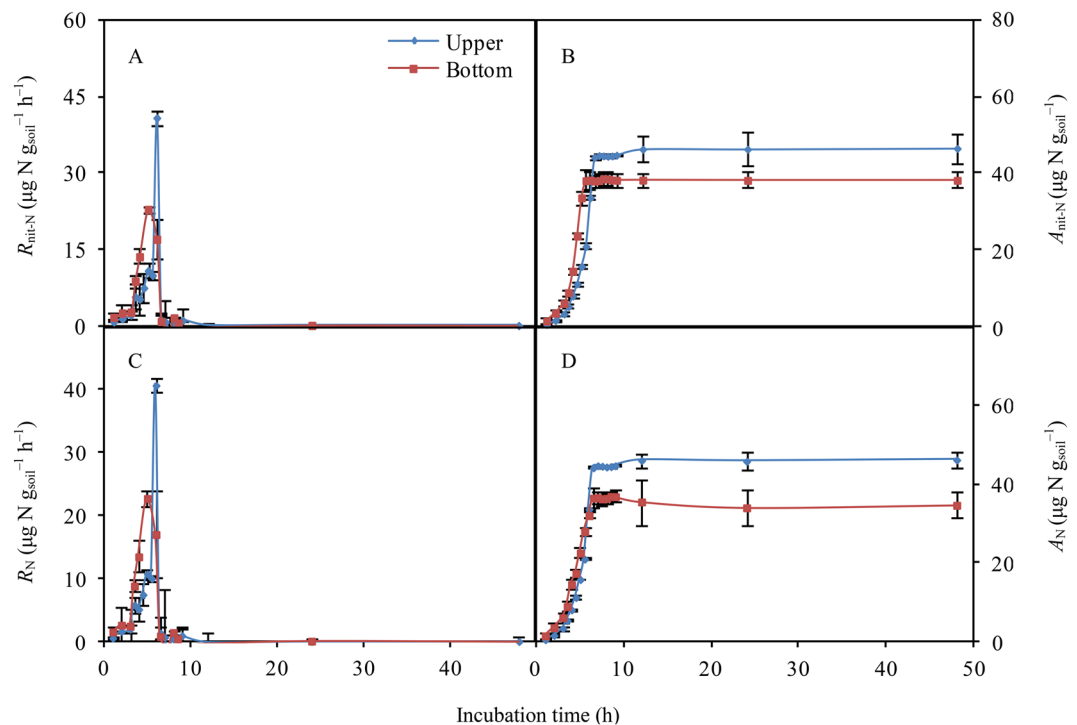


Figure 4. Responses of soil nitrogen mineralization and nitrification to rewetting, (A) soil nitrogen nitrification rate ($R_{\text{nit-N}}$, $\mu\text{g N g}_{\text{soil}}^{-1} \text{h}^{-1}$), (B) accumulation of $R_{\text{nit-N}}$ ($A_{\text{nit-N}}$, $\mu\text{g N g}_{\text{soil}}^{-1}$), (C) soil nitrogen mineralization rate (R_{N} , $\mu\text{g N g}_{\text{soil}}^{-1} \text{h}^{-1}$), (D) accumulation of R_{N} (A_{N} , $\mu\text{g N g}_{\text{soil}}^{-1}$).

Dynamics of R_{N} and pulse effects of rewetting. After rewetting, R_{N} first exhibited a stable increase, then a sharp increase, and finally a steep decrease in both the upper and bottom locations (Fig. 4). The results indicated that $R_{\text{nit-N}}$ was the main contributor to R_{N} , while the contribution of $R_{\text{amm-N}}$ was small ($0.01\text{--}0.65 \mu\text{g N g}_{\text{soil}}^{-1} \text{h}^{-1}$) and we therefore did not show it in Fig. 4.

Rewetting events also exerted strong pulse effects on R_{N} . The observed $R_{\text{N-max}}$ values were significantly higher in the upper location ($40.87 \mu\text{g N g}_{\text{soil}}^{-1} \text{h}^{-1}$) than that in bottom locations ($22.86 \mu\text{g N g}_{\text{soil}}^{-1} \text{h}^{-1}$), respectively ($P < 0.05$, Fig. 5A). $T_{\text{N-max}}$ values were observed at 6 and 5 h in the upper and bottom locations, respectively. $D_{1/2\text{max-RN}}$ values were estimated as 1.5 and 2.5 h for the upper and bottom samples, respectively. The values of A_{N} were calculated as $46.54 \mu\text{g N g}^{-1}$ (upper) and $38.25 \mu\text{g N g}^{-1}$ (bottom), which was significantly higher in the upper location ($P < 0.001$, Fig. 6). Similar to the R_{C} results, no significant relationships between R_{N} and MBN were observed from the start to peak R_{N} during the 48-h incubation period (data not shown).

Relationships between R_{C} and R_{N} through drying-rewetting-drying. No correlation between R_{C} and R_{N} was observed through the drying-rewetting-drying processes. Even dividing the incubation processes as the maximum of MBN, R_{C} and R_{N} were not coupled in the early or late phase of the maximum of MBN in the total 48-h incubation period (all $P_s > 0.05$, Figs S2, S3, S4).

Discussion

Strong and rapid pulse effects of rewetting on R_{C} . Rewetting exerted strong and rapid pulse effects on R_{C} in view of $R_{\text{C-max}}$ and $T_{\text{RC-max}}$. These pulse effects were observed immediately and peaked at about 10 min (Figs 1 and 2). The observed $R_{\text{C-max}}$ values were about 80 to 90 times to the initial R_{C} values under air-drying conditions. Birch.H.F.⁵ first reported that heavy precipitation may result in strong, short pulse effects on R_{C} and soil microbial growth in soils that have experienced long-term drought. Based on measurements of R_{C} after 15 and 30 min and 1, 2, 5, 24, and 48 h of simulated precipitation, Sponseller¹⁰ found that R_{C} responded immediately, peaked at 30 min, and returned to its original level within 48 h. Using a microorganism respiration apparatus at 25 °C with measurements at 3-h intervals, Griffiths²⁰ found that R_{C} increased after 12 h with a peak at 24 h. Based on this research, we inferred that the shorter, stronger responses of R_{C} have not been detected by previous studies, resulting in underestimation of the pulse effects of rewetting (or precipitation events). A related study of Jones and Murphy¹² indirectly supported the idea that the responses of soil microbes to simulated rainfall are significant in arable and grassland soil, with the greatest level of CO_2 production occurring after about 16 min. Some studies have demonstrated that after the pulse effects, the CO_2 release rate may return to its original level after 48 h, a longer period than that found in this study¹⁰. In this study, the optimal SWC (55% WHC) after simulated rewetting and temperature (22 °C), in part, should explain the observed stronger pulse effects. Furthermore, the pulse effects have been predicted to vary for different seasons, initial SWCs, and drying-wetting frequencies^{1,21,22}, factors which require further investigation.

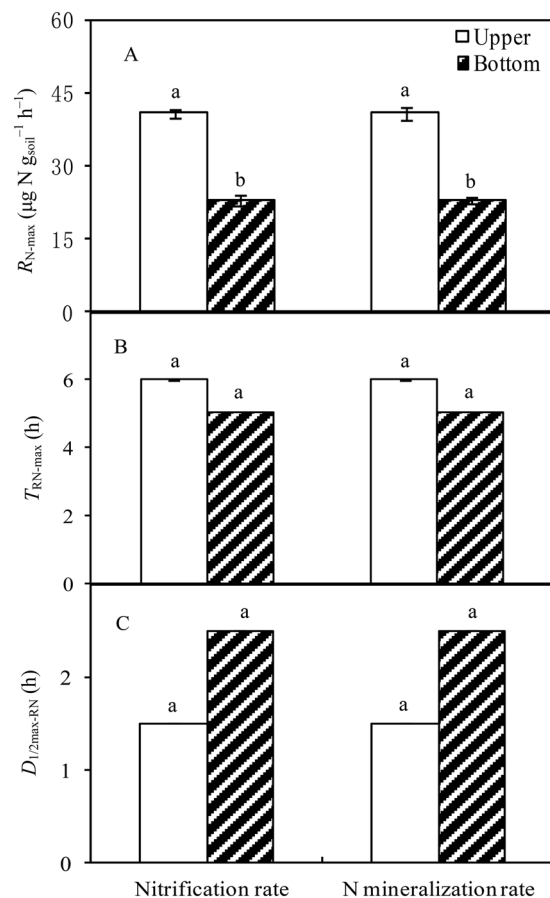


Figure 5. Pulse effects of soil nitrogen mineralization and nitrification from simulated rewetting event, (A) maximum soil nitrogen mineralization or nitrification rate (R_{N-max} , $\mu\text{g N g}_{soil}^{-1} \text{h}^{-1}$), (B) time to reach R_{N-max} (T_{RN-max} , h), (C) duration from $1/2 R_{N-max}$ to $1/2 R_{N-max}$ ($D_{1/2 max-RN}$, h).

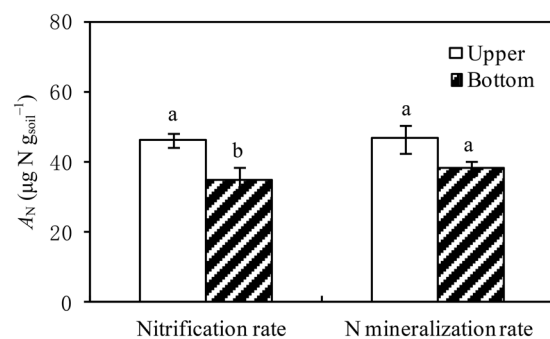


Figure 6. Accumulation of soil nitrogen mineralization and nitrification rates (A_N , $\mu\text{g N g}_{soil}^{-1}$) during the 48-h incubation period.

Strong and short-term pulse effects of rewetting on R_N . Rewetting exerted strong pulse effects on R_N , which rapidly increased after simulated rewetting, with peak soil inorganic N content occurring at 5–6 h. This apparently delayed R_N (Figs 5 and 6). Precipitation events or drying-wetting processes are the main drivers of the N supply for plant growth in arid and semiarid areas^{3,23}. In this study, the content of soil inorganic N may reach the levels 35–45 $\mu\text{g N g}_{soil}^{-1}$ after 5–8 h of rewetting, suggesting a capacity for soil inorganic N supply from the short-term pulse effects of small precipitation events.

Pulse effects depend on the size, frequency, and duration of rewetting (or precipitation events)¹. In arid and semiarid regions, most precipitation events are small^{24–26}. For example, Sala and Lauenroth²⁶ reported that precipitation events of 5 mm or less accounted for 70% of all precipitation events in a North American shortgrass steppe, while Zhang and Zhao²⁷ found that these small events comprised 82% of precipitation events in the Heihe River basin. The pulse effects of small precipitation events may play important roles in the available supply of nutrients

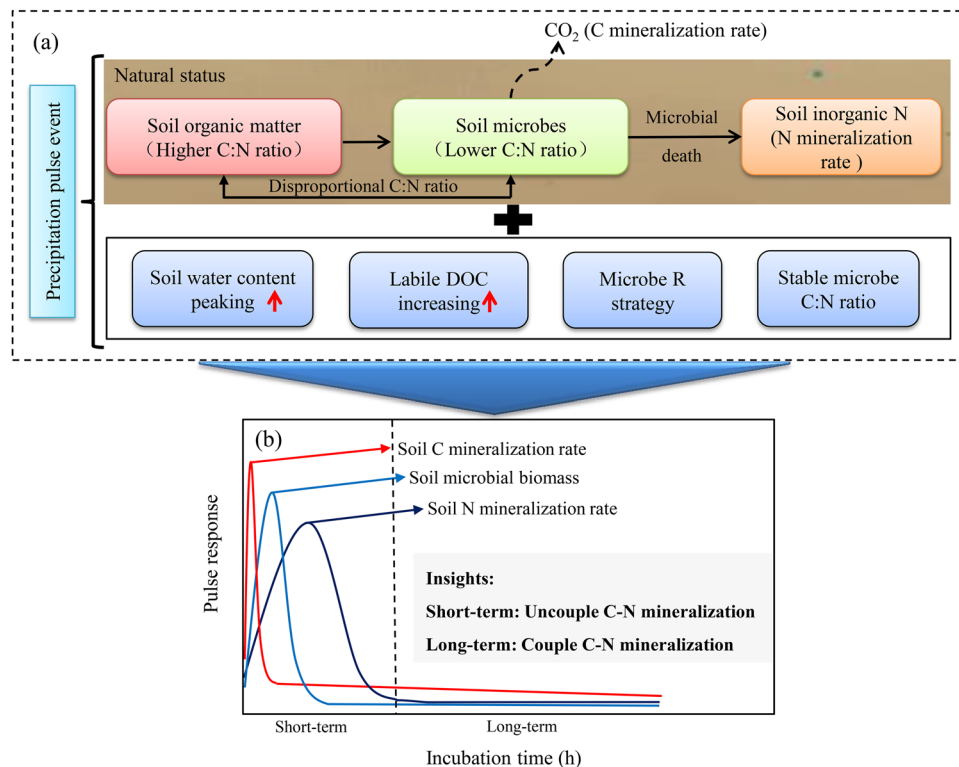


Figure 7. General concept of asynchronous pulse responses of soil carbon and nitrogen mineralization regulated by soil microbes to simulated rewetting.

in arid and semi-arid regions, especially for some plant species with rapid growth and reproductive strategies^{28,29}. McIntyre, *et al.*³⁰ concluded that riparian soils produced the greatest mineralization flush, as over 70% of the total mineralized N accumulated within 48 h of rewetting. Our findings provide direct evidence of short-term pulse effects of small precipitation events (rewetting) on R_N , and we infer that increased precipitation quantity (or intensity) may trigger stronger and longer pulse effects, which should depend on the initial water levels of soils to a large extent¹. An understanding of the dependence of pulse effects on precipitation intensity will be necessary for future explorations of biogeochemistry and the interactions between soil and plants in arid and semiarid regions. Furthermore, it is necessary to mention that our findings were concluded from one cycle of rewetting event, so our findings could be valid for further cycles, only if the pulse effect is maintained, which have been demonstrated in previous studies that dry-rewetting cycles regulate plant C rhizodeposition, stabilization and N cycling²⁹⁻³¹. However, the substrate depletion and changes in soil microbial composition are inevitable in several cycles of rewetting events, resulting in a variable Birch effects (especially for intensity vs. duration). Therefore, experiments considering more cycles of rewetting events are required in future.

Uncoupled pulse responses of R_C and R_N driven by soil microbes. Under microbial regulation, the pulse responses of R_C and R_N were asynchronous and uncorrelated. Our results support the assumption that the rapid growth of microbes and the microbial requirement of C:N stoichiometric stability^{32,33} may result in uncoupled C and N mineralization through the short-term pulse effects of rewetting (or simulated precipitation). These findings differ from those of previous studies conducted under normal SWC conditions, which found that R_C and R_N were closely coupled in natural ecosystems¹³, and incubation experiments at weekly or monthly intervals^{14,15}.

Through the drying-rewetting process to stimulate a precipitation events (Fig. S1), soil microorganisms play important roles as reservoirs of soil nutrients and regulators of soil C and N mineralization rates^{1,16}, resulting in apparently asynchronous R_C and R_N processes and the observed uncoupled relationships. We developed a conceptual framework to explain the asynchronous pulse responses of R_C and R_N to simulated rewetting, regulated by soil microbes (Fig. 7). In detail, precipitation event rapidly enhance SWC and the availability of dissolved organic C, and therefore promote a sharp increase in soil microbial biomass (Fig. 2). Simultaneously, the higher C:N ratios of SOM and lower C:N requirements of soil microbes lead the microbes to decompose more SOM to satisfy their N requirement. This results in a sharp increase in CO₂ emissions as surplus elements due to the relative stability of the C:N ratios in soil microbes. According to classical homeostasis theory in ecological stoichiometry, organisms have the capacity to maintain a stable C:N:P ratio despite differences in the availability of these elements^{32,34,35}. Well-constrained C:N:P ratios of soil microbes have been reported recently using global meta-analysis³³. With the rapid growth of soil microbes, the depletion of labile DOC and decreasing soil moisture result in an increasing microbial death and an apparent decrease in soil microbial biomass (supported by a sharp decrease in MBN shown in Fig. 2), and therefore, we can observed a rapid increase in soil N mineralization, accompanied by a steep

Position	Location	Altitude (m.a.s.l.)	pH	Soil carbon content (%)	Soil nitrogen content (%)	C/N ratios
Upper	39.9901°N, 116.1717°E	500.1	8.14 ± 0.02 ^a	2.26 ± 0.01 ^a	0.19 ± 0.01 ^a	12.06 ± 0.34 ^a
Bottom	39.9667°N, 116.2086°E	21.2	7.56 ± 0.01 ^b	3.40 ± 0.01 ^b	0.20 ± 0.01 ^a	17.16 ± 0.53 ^b
F			5.57	0.07	0.23	1.59
P			<0.001	<0.001	0.30	<0.05

Table 1. Selected soil properties of the experimental plots. †Data are represented as means ± 1 SD (n = 3). Data with same superscript letters within each column indicated no significant difference at P = 0.05 level.

decrease in soil C mineralization³⁶. After several hours of precipitation, the C:N requirements of soil microbes and microbe biomass stabilize, and coupled soil C and N mineralization relationships may be observed¹⁹. Overall, the shifting roles of soil microbes from reservoirs to releaser for N may exemplify the key role of microbes in soil C and N mineralization during different phase, and which can explain the uncoupled relationships among R_N , R_C , and MBN at the initial phase of precipitation events. It is necessary to point out that, in this study, the data of MBC were not measured. Therefore, we assumed that MBN should be a proxy of MBC and microbial biomass some extent because of the stoichiometric homeostasis assumption of stable MBC:MBN ratio.

Rewetting events (or simulated precipitation) exert rapid, strong pulse effects on soil C and N mineralization rates over the short term. Microbes drive asynchronous processes in soil C and N mineralization rates and the uncoupled relationships among R_N , R_C , and MBN. This research provides a new conceptual framework of the pulse effects of rewetting (or small precipitation events) on soil C and N mineralization rates, as well as insights into the importance of these pulse effects in biogeochemistry and the interaction of plants and soils in arid and semiarid regions. It is necessary to mention that these findings were derived only from one incubation experiment, and therefore validation with more incubation experiments and field experiments are required in future.

Materials and Methods

Study area. Soil samples were collected from temperate forests northwest of Beijing (39° 24'N, 116° 10'E). This region has a semi-arid temperate continental monsoon climate. The annual average temperature is approximately 11 °C, ranging from −1.4 °C in January to 22.4 °C in July. The annual average precipitation is about 626 mm. The regional soils are classified as Lixisols, according to the classification of world reference base for soil resources³⁷. The dominant tree species are pine (*Pinus*) and maple (*Liquidambar formosana*). We established three sampling plots at two different elevations, designated as bottom (21.2 m.a.s.l.) and upper (500.1 m.a.s.l.). Selected soil properties of the two locations are provided in Table 1.

Soil sampling and chemical analyses. Soil sampling was conducted in early November 2014. We randomly established three plots (10 × 10 m) at each location (bottom and upper) and collected 15 to 20 surface soil samples (0–10 cm) from each plot using a soil auger of 5 cm in diameter. The soil samples were sieved (< 2 mm), and roots and visible organic debris were removed by hand. Approximately 3 kg of each soil sample were air-dried for chemical analysis and incubation experiments.

The concentrations of soil C and N were measured using an elemental analyzer (vario MAX cube, ELEMENTAR, Germany). Soil pH was measured in a soil-water slurry (1:2.5, w/w) with an Ultrameter-2 pH meter (Myron L. Company, California, USA). Soil water holding capacity (WHC, %) and gravimetric water content (SWC, %) were measured using the interior-ringer cutting and oven-drying weight.

Incubation experiments and measurements of soil C and N mineralization rates. Soil samples were first air-dried at room temperature for approximately one week until SWC was less than 10% WHC. Ten-gram samples of air-dried soil were placed in incubation bottles (5 cm in diameter, 10 cm in height), with three replicates for each location. Basic soil respiration rates were measured at 22 °C (as detailed in section 4.3.1), and soil inorganic N content was measured (as detailed in section 4.3.2). Then, all soil samples were rewetted to 55% WHC (with approximately 2.5–3.1 mm of simulated precipitation) and incubated at 22 °C^{38–40}. During the 48-h incubation period, R_C was continuously measured at 6-min intervals at 22 °C⁴¹. Furthermore, the SWC (%) of samples in the incubation bottles was measured at 0, 1, 2, 3, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 12, 24, and 48 h to determine the effects of drying-rewetting-drying (Fig. S1).

Similarly, ten-gram samples of air-dried soil were placed in incubation bottles (5 cm in diameter, 10 cm in height), with 57 replicates for each location, which were used for 19 separate destructive sampling times to measure the content of inorganic N (NO_3^- and NH_4^+) and MBN.

Measurement of R_C . A continuous-measurement apparatus developed by He, *et al.*³⁸ and Dai, *et al.*⁴² was used to measure the R_C of each sample at 6-min intervals for 48 h. R_C was calculated using the slope of CO_2 concentration and conversion factors^{38, 43, 44}, as follows:

$$R_C = \frac{c \times v \times \alpha \times \beta}{m} \quad (1)$$

where R_C is soil microbial respiration rate ($\mu\text{g C g}_{\text{soil}}^{-1} \text{h}^{-1}$), C is the slope of the change in CO_2 concentration, V is the volume of the incubation bottle and gas tube, m is the soil weight (g), α is the conversion coefficient for CO_2 mass, and β is a conversion coefficient of time. Here, V and α are used to transform volume concentration to mass

concentration, and β is used to transform the measuring time from second to hour or day because the record of Licor 7000 is setup on per second basis.

Measurement of R_N and MBN. The contents of soil ammonium ($\text{NH}_4^+\text{-N}$) and nitrate ($\text{NO}_3^-\text{-N}$) were measured before and after incubation at intervals of 0, 1, 2, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 12, 24, and 48 h (3 replicates). In brief, 10-g soil samples were mixed with 25 mL of 0.5 M K_2SO_4 solution, shocked for 1 h, and then measured $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ with a filtered solution^{45,46}. $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and TN concentrations were determined with a flow analyzer (FUTURA, Alliance Instruments, France).

R_N ($\mu\text{g N g}_{\text{soil}}^{-1} \text{h}^{-1}$) was calculated using the following equations:

$$\Delta t = t_{i+1} - t_i \quad (2)$$

$$R_{\text{amm}} = L \times c[\text{NH}_4^+ - \text{N}]_{i+1} - L \times c[\text{NH}_4^+ - \text{N}]_i \quad (3)$$

$$R_{\text{nit}} = L \times c[\text{NO}_3^- - \text{N}]_{i+1} - L \times c[\text{NO}_3^- - \text{N}]_i \quad (4)$$

$$R_N = R_{\text{amm}} + R_{\text{nit}} \quad (5)$$

where t_i and t_{i+1} are the initial and ending times of incubation, respectively; Δt is the duration of incubation; $c[\text{NH}_4^+\text{-N}]_i$ and $c[\text{NH}_4^+\text{-N}]_{i+1}$ are the concentrations of $\text{NH}_4^+\text{-N}$ before and after incubation, respectively; $c[\text{NO}_3^-\text{-N}]_i$ and $c[\text{NO}_3^-\text{-N}]_{i+1}$ are the concentrations of $\text{NO}_3^-\text{-N}$ before and after incubation, respectively; L is the volume of solution leached; and R_{amm} , R_{nit} , and R_N are the $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and total N mineralization rates on basis of per g dry soil, respectively.

Additionally, the contents of MBN were measured using the chloroform fumigation extraction method at intervals of 0, 1, 2, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 12, 24, and 48 h after rewetting, and each treatment has three replicates. In brief, the measurements were conducted using destructive sampling with the same treatments as in the measurements of R_N . The soil samples were divided into two groups: 24-h fumigated (chloroform) and un-fumigated. Microbial biomass N ($\mu\text{gN g}_{\text{soil}}^{-1}$) was calculated as the difference in N extracted from the fumigated and un-fumigated samples under 1-h shake⁴⁷.

Characterizing the pulse effects of rewetting on R_C and R_N . The following four parameters were selected to describe the pulse effects of rewetting on R_C and R_N : (1) the maximum rates of R_C and R_N ($R_{C\text{-max}}$ and $R_{N\text{-max}}$, respectively); (2) the times to reach $R_{C\text{-max}}$ and $R_{N\text{-max}}$ ($T_{R_{C\text{-max}}}$ and $T_{R_{N\text{-max}}}$, respectively); (3) the duration of pulse effects from the start to the end of one-half $R_{C\text{-max}}$ and $R_{N\text{-max}}$ ($D_{1/2\text{max-}R_C}$ and $D_{1/2\text{max-}R_N}$, respectively); and (4) the accumulation of R_C and R_N during the 48-h incubation period (A_C and A_N , respectively).

Statistical analyses. Data were represented as mean and standard deviation (SD). T -tests were used to identify significant differences in soil properties between samples from the bottom and upper locations and the pulse effects (R_{max} , $T_{R\text{-max}}$, $D_{1/2R\text{max}}$, and A). Regression analyses were used to evaluate the relationships between R_C , R_N , and MBN. Statistical analyses were performed using SPSS 13 (SPSS Inc., Chicago, IL, USA). The significance level was defined as $P = 0.05$.

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Author Contributions

S.X. collected and analyzed the data and wrote the manuscript. H.N. and W.C. supervised the project and commented on the contents of the manuscript. Z.J., H.N., H.J., T.J., Z.X. and L.Y. conducted the data pre-processing. H.N. and Z.J. revised and edited the manuscript.

Additional Information

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