



Original article

Short-term responses of soil enzyme activities and carbon mineralization to added nitrogen and litter in a freshwater marsh of Northeast China



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ABSTRACT

Soil organic matter decomposition is regulated by nutrient availability. Adding nitrogen (N) and litter could affect the stability of soil organic carbon (SOC). An incubation experiment was conducted to examine the effects of ammonium nitrate (AN), urea (U), and litter amendment on activities of soil microbial extracellular enzyme, soil microbial biomass C (MBC), dissolved organic C (DOC), and C mineralization in freshwater marsh. The results showed that adding N including AN and U decreased soil urease activity, MBC, DOC, and soil pH. However, litter amendment and combined litter and N amendment increased the enzymes activities of urease and invertase, MBC and DOC concentrations. The response of soil C mineralization to N and litter additions was different, being inhibited with AN addition while stimulated with litter addition and the combined litter and N addition, initially stimulated but inhibited thereafter with urea addition. Our results suggest that adding N is helpful for improving marshland soil C stocks via decreasing soil cumulative CO₂-C emissions, labile organic carbon concentration of MBC and DOC. However, adding litter could reduce C storage stability by stimulating soil C mineralization and increasing soil labile C fractions and enzyme activities.

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1. Introduction

Freshwater marsh plays an important role in the global carbon (C) cycle. The increase in the atmospheric nitrogen (N) deposition due to human activities is one of major concerns in marshland ecosystems. The stability of soil organic C (SOC) strongly depends on the input of N to the soil and availability of litter because soil microbial decomposition processes are greatly influenced by C and N availability [22,32]. The addition of C and N can influence the soil C mineralization rate, microbial biomass, and enzymatic activities. In peatlands, N deposition favored microbial decomposition by removing N constraints on microbial metabolism and having a positive feedback on microbial enzymatic activities through a chemical amelioration of litter peat quality [2]. Thus, to understand how N deposition affects soil C decomposition is crucial to evaluate the response of C cycling to environmental changes in marshland ecosystems.

The effects of N deposition on soil decomposition processes depend on the forms of N used and the time scale of the study. Both inorganic and organic N additions could influence soil organic matter (SOM) decomposition [11,19,21]. Short-term ammonium nitrate (AN) input has a negative effect on SOM decomposition and enzyme activities [21] and has a positive effect on N mineralization [23]. During long-term experiments, it is found that adding AN had positive, negative, and neutral effects on the SOM decomposition rates, primarily depending on the activities of extracellular enzymes [16]. Urea (U) is another form of N addition that often used in the field experiments. It has shown that U addition has a much more profound effect on the SOM than either ammonium or nitrate. It has been also observed that urea-amended soils appear to change microbial components [11], stimulate microbial C cycling, enhance soil fluxes of CO₂, and accelerate the mineralization of SOC during short-term incubations [24]. Long-term U addition increased the turnover of SOC, but it also led to significantly enhanced C input to soil, which overcame the increase in SOC turnover and subsequently increase the soil C concentrations [12]. However, it also found that long-term U addition tended to stabilize organic carbon in both whole soil and some soil fractions [29].

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Plant residue improves soil quality by increasing the microbial biomass C (MBC), in particular, labile plant residue acts as a C or N source for microorganisms, accelerating their decomposing activity [1]. Nitrogen deposition can change nutrient uptake and plant photosynthetic efficiency, ultimately controlling the quantity and biochemistry of litter inputs to the soil [28]. Introducing this additional substrate to the soil have shown a positive effect on C mineralization by increasing MBC, dissolved organic C (DOC), and enzyme activities in the early stage of incubation [5]. In fact, SOC turnover associated with N and litter addition is complex. However, little research has been done about the effect of litter addition on SOC decomposition and their relationships with N addition, especially in wetland soils.

Soil enzymes play an important role in organic matter decomposition and nutrient cycling since enzymes as the key players involved in the biochemical catalytic reactions. Ref. [7] proposed that the low rate of biodegradation in peatlands is primarily due to oxygen constraints on phenol oxidase activity, leading to phenolic materials accumulation and inhibition these pivotal hydrolase enzymes. Enzyme activities and their responses to added N and litter have received considerable attention. Ref. [5] found that soil enzyme activities and microbial biomass increased in the litter-amended soils during the first stage of incubation but decreased thereafter. Residue incorporation into soils can strongly affect soil microbial activities by altering the contact between soils and residues, and thus modifying microbial enzymatic synthesis and reactions [18]. Soil N availability is another important factor that regulates the competitive interaction of soil microorganisms, thereby modifying the relative production of soil enzymes. The effects of adding N on soil enzyme activities can be triggered by the selective pressure of N on soil microbes [13]. Studies in wetland ecosystem are necessary to evaluate the influences of both N and litter addition on soil enzyme activities, and will be helpful for clarifying the mechanism of the variability of SOM.

The objective of this study was to examine how soil C mineralization responds to the N addition or in combination with *Calamagrostis angustifolia* litter input in terms of MBC, DOC and extracellular enzyme activities in marshland. We speculated that the stability of soil C stocks would be proportional to the changes in soil labile C fractions and extracellular enzyme activities induced by N and litter addition; adding N including AN and U would increase soil cumulative $\text{CO}_2\text{-C}$, MBC and DOC because of the elevated soil N availability; and a stimulated effect of the added litter on soil C mineralization would be presented due to the provided C substrate for soil microorganisms.

2. Materials and methods

2.1. Soil and litter collection

In May 2011, soils for the experiments were collected from 0 to 20 cm layer of a freshwater marshland in the Sanjiang Mire Wetland Experimental Station, Chinese Academy of Sciences (47°35'N, 133°31'E) in Sanjiang Plain, Northeast China (Fig. 1). The annual average temperature is 2.5 °C with growing season duration of 125 d. The annual precipitation in this area is 550–600 mm, of which more than 60% falls from June to August. The dominant species is *C. angustifolia*. The collected soil samples were brought to the laboratory. The green material and roots were removed. Then, the samples were passed through a 2-mm sieve, mixed thoroughly, and divided into two subsamples. One subsample was used for the laboratory incubation experiment, while the other was used to determine the initial soil properties. The above-ground litter in the sampling site was sampled before the soil samples were collected. Following air drying and grinding, the plant materials were divided

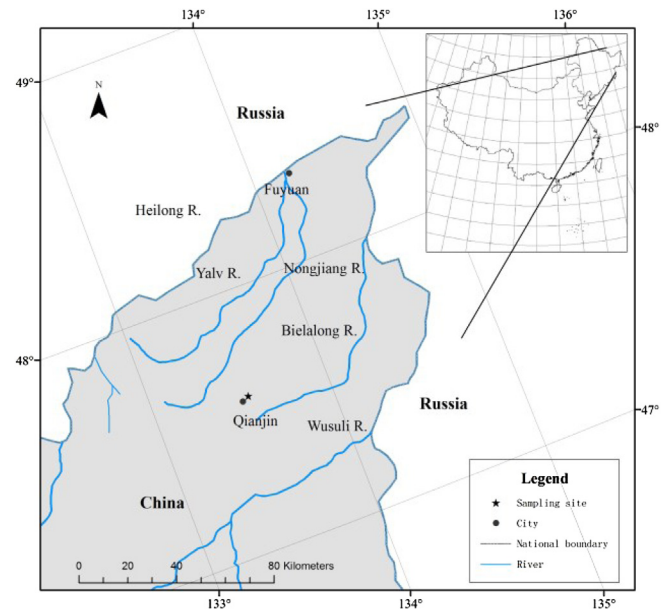


Fig. 1. Location of sampling site in the Sanjiang Plain of Northeastern China.

into two subsamples. One subsample was used for the incubation experiment. The other was used to determine the initial litter properties. Selected properties of the soil and litter are listed in Table 1.

2.2. Experimental design and treatments

Fresh soil samples (100 g dried weight) were placed in 500 ml glass jars and pre-incubated for 3 days. For the N treatment, AN and U solutions at a rate of 200 mg N kg^{-1} of soil were separately added, and the soil moisture content were adjust to 100% of soil water holding capacity by deionized water. Meanwhile, deionized water was added into the soils as the control. For the litter addition treatment, 2.0 g of litter was mixed thoroughly with the soil samples before the N solution was added. Therefore, six treatments were used and named according to the added substance, namely control (CK), ammonium nitrate (AN), urea (U), litter (L), litter and ammonium nitrate (LAN), and litter and urea (LU). Each treatment has three replicates. All glass jars were covered with polyethylene film and were punctured with needle holes to maintain aerobic conditions. All the glass jars were incubated at 20 °C for 120 days. The weight of each sample was recorded at the beginning of the treatment, and water loss from evaporation was supplemented with deionized water in every five days. Three replicates per treatment group were destructively sampled to determine the soil urease and invertase activities, MBC, DOC, ammonia ($\text{NH}_4^+\text{-N}$), and nitrate ($\text{NO}_3^-\text{-N}$) concentrations at the days of 15, 30, 60, 90, and 120.

2.3. Soil C mineralization

Soil C mineralization was periodically determined during the incubation periods by measuring the CO_2 concentration to present the biological indices of SOM stability. Soil CO_2 efflux was determined on the days of 1, 3, 6, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 110, and 120 during the incubation periods. Each glass jar was sealed air tightly and allowed to accumulate CO_2 for 1 h. The headspace gas was drawn using a 50 ml syringe equipped with a three-way stopcock. The collected gas was detected by a gas

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