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# Distribution and mineralization of organic carbon and nitrogen in forest soils of the southern Tibetan Plateau

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## ABSTRACT

The forests of the Tibetan Plateau store large amounts of soil organic carbon (OC) but are among the most vulnerable and sensitive ecosystems to environmental change. The lack of knowledge regarding the distribution and turnover of OC and nitrogen (N) in Tibetan Plateau forest soils limits the ability to predict how this ecosystem will respond to climate change. In this study, we collected mineral soils from coniferous and broadleaf forests on the southern Tibetan Plateau and measured OC and N contents in both bulk soils and water-stable aggregates. We also determined the mineralization of OC and N in bulk soils and examined the effects of N addition on OC mineralization. Our objectives were to investigate the distribution and mineralization of soil OC and N in various forest types and to determine the stability of OC following N addition. Our results showed that OC and N in macroaggregates contributed 76% of the OC and N mineralization and their relationships with soil OC or N contents were similar between broadleaf and coniferous forests, indicating that soil OC and N distribution and turnover were insensitive to forest type. Nitrogen mineralization was dominated by ammonification in these forest soils. Nitrogen addition did not affect OC mineralization or its relationship with soil OC or N contents. These results indicate that the OC contents of forest soils of the southern Tibetan Plateau are relatively insensitive to N addition.

## 1. Introduction

The Tibetan Plateau is a vast elevated plateau in Asia and is the world's highest and largest plateau. The forests on the plateau occupy an area of 0.62 million km<sup>2</sup> (Deng, 2000) and have large stocks of soil organic carbon (OC) (Yang et al., 2014), particularly in the southern part of the plateau (Jiang et al., 2009). However, the distribution and turnover of soil OC and nitrogen (N) in the forests of the plateau have not been well characterized, and ecosystem processes in this region are therefore poorly understood. Furthermore, the ecosystems on the Tibetan Plateau are sensitive to environmental changes such as global warming and N addition. On the Tibetan Plateau, the effects of warming have mostly been studied in grassland ecosystems (Zhang et al., 2015), and few studies have examined forest ecosystems (Xu et al., 2010); the effects of N addition have rarely been studied in either ecosystem. This represents an important gap in our understanding of how these Tibetan Plateau ecosystems will respond to global environmental change.

Most of the soil OC in forests is occluded within aggregates and thereby physically protected from microbial decomposition (von Lützow et al., 2007; Wei et al., 2013). The OC and N contents are assumed to increase with aggregate size based on the hierarchical aggregation theory (Elliott, 1986), and changes in the OC content of bulk soils are linearly related to changes in the aggregate-associated OC content (Qiu et al., 2015; Spohn and Giani, 2011). For example, results from the Loess Plateau show that the accumulation of OC in bulk soils following the conversion of farmland to forests was mainly due to the accumulation of OC in macroaggregates (Qiu et al., 2015). The distribution of OC and N among aggregates thus determines the dynamics of OC and N in forest soils. However, the distribution of soil OC and N among aggregates in the forest of the Tibetan Plateau has not previously been tested.

Forest type determines the quality and quantity of litter input and thus the composition and biodegradability of soil organic matter (Augusto et al., 2002, 2015; Cools et al., 2014; Mueller et al., 2012). Coniferous needles generally have more recalcitrant C components than broad leaves

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(Shirato and Yokozawa, 2006), resulting in a lower OC and/or N mineralization rate. For example, the *in situ* net N mineralization rate in spruce soils was found to be one quarter the rate in beech soils in temperate German forests (Schütt et al., 2014). A recent study in the temperate forests of northern China also showed that forest type affected the coupled relationships of OC and N mineralization (Quan et al., 2014). However, whether broadleaf forests of the Tibetan Plateau have higher OC and N mineralization than coniferous forests is unknown.

As one of the most important factors in global change, N deposition is assumed to significantly affect soil OC mineralization due to its effects on the chemical properties of soil, microbial and enzymatic activity, and substrate quantity and availability (Hobbie, 2008; Min et al., 2011). Nitrogen addition has been reported to increase, have no effect on, or decrease soil OC mineralization, depending on the condition of soil OC and N, the quality of soil organic matter, and soil properties (Hagedorn et al., 2012; Knorr et al., 2005; Wang et al., 2014). For example, Janssens et al. (2010) and Liu and Greaver (2010) reported decreased heterotrophic respiration following N addition, while Menvailo et al. (2014) and Tu et al. (2013) found that N addition led to increased OC mineralization in forest soils due to a decrease in the C:N ratio and a shift in the ratio of bacterial to fungal biomass. This uncertainty in the response of ecosystem processes to global change factors highlights the necessity for further examination of the effects of N addition on OC mineralization, particularly in the Tibetan Plateau.

We hypothesize that in forest soils of the Tibetan Plateau, (H1) macroaggregate-associated OC and N dominate soil OC and N; (H2) soils in broadleaf forests have higher OC and N contents and mineralization than coniferous forests, because broadleaf forests tend to have more labile carbon than coniferous forests; and (H3) N addition will increase soil OC mineralization because N addition decreases the soil C/N ratios and thus stimulates mineralization. To test these hypotheses, we measured the distribution and mineralization of OC and N in soils collected from coniferous and broadleaf forests on the southern Tibetan Plateau. The main objective of this study was to evaluate how forest types and N deposition affect the OC and N distribution and stabilization in soils, which have not been assessed in the region of Tibetan Plateau, but are essential to understand given the role of soil OC and N in constraining the effects of global change on terrestrial ecosystems.

#### 2. Materials and methods

#### 2.1. Study site and soil sampling

Soil samples were collected from forests near the town of Zhangmu in Nyalam County, Tibetan Autonomous Region, China (27°59.54'N, 85°58.44'E), at an altitude ranging from 1700 to 2300 m a.s.l. The study site is located in the southern part of the Tibetan Plateau and has a subtropical monsoon climate. The mean annual temperature is 20 °C, and the mean annual precipitation is 2500 mm. Rain falls mainly between March and October. The soil is yellow to brown in color and is classified as a Ferric/Haplic Luvisol in the FAO/UNESCO system, with a sandy loam texture. The soil is free of carbonates. Vegetation in the area consists of mature broadleaf and coniferous forests.

At two sites that were approximately 3 km apart, three adjacent transects in broadleaf and coniferous forests were selected for soil sampling in October 2011. At these sites, the broadleaf forest was dominated by oak (*Quercus semecarpifolia* Smith) and birch (*Betula alnoides* Buch.-Ham. ex D. Don), and the coniferous forest was dominated by fir (*Abies georgei* var. *smithii*) and spruce (*Picea likiangensis* var. *1inzhiensis*). Three plots ( $20 \times 20$  m) were established in each transect. The distance between each plot was > 100 m. In both forest types, the selected plots had the same soil type and similar physiographic conditions and slope gradients. Therefore, the effects of land surface processes (*e.g.*, runoff) on soil properties were similar, and that the differences in soil OC and N distribution and turnover were mainly due to the variations in forest types. In each plot, five subplots were randomly established. Soil samples were

collected from depths of 0–10 and 10–20 cm using a soil-coring kit (5.0 cm in diameter) at each of the five subplots. These samples were combined into a composite sample for each plot. The organic layer was removed before sampling. Visible pieces of organic material were removed, and the moist field soil samples were transported to the laboratory, air-dried and stored at 4 °C for 3 days prior to analyzing the aggregate size distribution, the OC and N contents in bulk soils and aggregates, and OC and N mineralization. The ways in which soil samples were transported and stored might have impacts on laboratory analyzing results (Rinkes et al., 2013). However, we tried to minimize the effects of samples handling by analyze all soils with the same method for each metric and, therefore, the results are comparable in this study.

#### 2.2. Laboratory measurements

Water-stable aggregates were isolated by wet sieving through 0.25and 0.053-mm sieves following the procedures described by Cambardella and Elliott (1993). The macroaggregates (> 0.25 mm), microaggregates (0.25–0.053 mm), and the silt + clay size class (< 0.053 mm) were oven dried at 50 °C for 24 h, weighed, ground to pass through a 0.25 mm sieve and then stored for OC and N analysis. A subsample of the air-dried, undisturbed soil or aggregate sample was ground to pass through a 0.25mm sieve for measuring the OC and N contents. The OC content was measured using the Walkley-Black method, and the N content was measured using the Kjeldahl method (Page, 1982).

To determine soil OC and N mineralization, 10 g of bulk soils were adjusted to 60% of the field-moisture capacity in 250-mL jars and preincubated for five days to remove the flush of C mineralization caused by re-wetting. The samples were then incubated in the dark at 25 °C. A 10-mL glass vial containing 5 mL of 1 M NaOH was placed in each jar to trap the released CO2. Soil moisture during the incubation was controlled by adding deionized water to maintain the initial weight. Six control jars were prepared with no soil samples. The amount of CO<sub>2</sub> trapped was determined by titration against 0.5 M HCl. CO<sub>2</sub> emission was measured at 4, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, and 85 days of incubation. At each sampling, the vials were replaced with another set of vials containing fresh NaOH, and the jars were returned to the incubator. The caps of the jars were opened periodically during sampling to replenish the oxygen supply in the headspace. The cumulative OC mineralized ( $C_{min}$ , in  $g kg^{-1}$  soil) after 85 days of incubation was calculated as the sum of OC mineralized at each sampling. After 85 days of incubation, the incubated samples were extracted with 2 M KCl with a soil to solution ratio of 1:10. The extracts were analyzed immediately for ammonium  $(NH_4^+)$  and nitrate  $(NO_3^-)$ using a Lachat Flow Injection Analyzer (AutoAnalyzer3-AA3, Seal Analytical, Norderstedt, Germany). The NH4<sup>+</sup> and NO3<sup>-</sup> contents of the soils before incubation were also measured using the same method.

To investigate the effects of N addition on OC mineralization,  $NH_4NO_3$  was added to 10 g of total soil at a rate of 0.1 g N kg<sup>-1</sup> soil, equivalent to a deposition rate of 10 g N m<sup>-2</sup> y<sup>-1</sup> (calculated by assuming a bulk density of 1.0 g cm<sup>-3</sup> and a soil depth of 10 cm for a mature forest). The soils with added N were then incubated for the determination of OC mineralization following the same procedure described above. Nitrogen mineralization was not determined for the soils with added N.

#### 2.3. Data analysis

The dynamics of OC mineralization were fitted to the following first-order model:

$$C_{t} = C_{p} \times (1 - e^{(-k \times t)})$$

$$\tag{1}$$

where  $C_t$  is the cumulative OC mineralized (g kg<sup>-1</sup> soil) at time t,  $C_p$  is the potentially mineralizable OC (g kg<sup>-1</sup> soil), and k is the first-order rate constant (d<sup>-1</sup>).

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