



## Consistent effects of canopy vs. understory nitrogen addition on the soil exchangeable cations and microbial community in two contrasting forests



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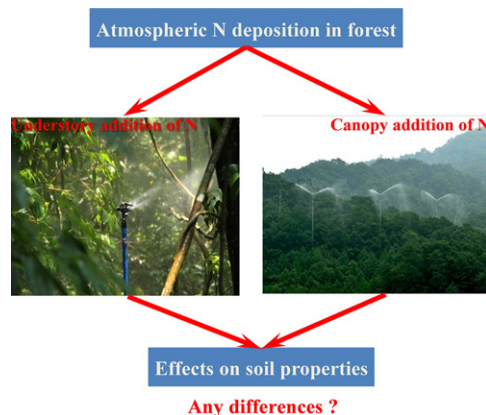
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### HIGHLIGHTS

- A novel field N manipulation experiment with both UAN and CAN was established.
- N addition reduced pH, BS and Ca and increased Al at temperate forest.
- N addition reduced biomasses of most soil microbial groups at subtropical forest.
- Effects of CAN on forest soils were not significantly different from that of UAN.
- No interactions between N treatment approach and study site or N addition rate.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Anthropogenic N deposition has been well documented to cause substantial impacts on the chemical and biological properties of forest soils. In most studies, however, atmospheric N deposition has been simulated by directly adding N to the forest floor. Such studies thus ignored the potentially significant effect of some key processes occurring in forest canopy (i.e., nitrogen retention) and may therefore have incorrectly assessed the effects of N deposition on soils. Here, we conducted an experiment that included both understory addition of N (UAN) and canopy addition of N (CAN) in two contrasting forests (temperate deciduous forest vs. subtropical evergreen forest). The goal was to determine whether the effects on soil exchangeable cations and microbial biomass differed between CAN and UAN. We found that N addition reduced pH, BS (base saturation) and exchangeable Ca and increased exchangeable Al significantly only at the temperate JGS site, and reduced the biomass of most soil microbial groups only at the subtropical SMT site. Except for soil exchangeable Mn, however, effects on soil chemical

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Soil microbial community  
Soil exchangeable cations

properties and soil microbial community did not significantly differ between CAN and UAN. Although biotic and abiotic soil characteristics differ significantly and the responses of both soil exchangeable cations and microbial biomass were different between the two study sites, we found no significant interactive effects between study site and N treatment approach on almost all soil properties involved in this study. In addition, N addition rate (25 vs. 50 kg N ha<sup>-1</sup> yr<sup>-1</sup>) did not show different effects on soil properties under both N addition approaches. These findings did not support previous prediction which expected that, by bypassing canopy effects (i.e., canopy retention and foliage fertilization), understory addition of N would overestimate the effects of N deposition on forest soil properties, at least for short time scale.

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## 1. Introduction

There is a growing consensus that increased atmospheric nitrogen (N) deposition due to human activities has emerged as one most serious global change problem (Vitousek et al., 1997; Galloway et al., 2008; Schlesinger, 2009; Pardo et al., 2011; Penuelas et al., 2013). At the global scale, the average rate of atmospheric N deposition has recently been estimated to be 105 Tg N yr<sup>-1</sup> (Galloway et al., 2008), and this rate is predicted to continually increase in the future (Dentener et al., 2006; Stocker et al., 2013). Furthermore, N deposition is especially serious in some “hot spots” such as central and southeastern China (Liu et al., 2013; Jia et al., 2014). Enhanced N deposition has been well documented to cause a series of detrimental effects, such as decline in plant diversity (Bobbink et al., 2010) and soil acidification (Tian and Niu, 2015), on terrestrial ecosystems.

Nitrogen deposition exerts profound effects on soil abiotic properties (Lucas et al., 2011; Tian and Niu, 2015) and biotic properties (Frey et al., 2004; Treseder, 2008). Many studies have indicated that soil chemical processes, such as soil acidification, are sensitive to N deposition (Gundersen and Rasmussen, 1990; Högberg et al., 2006; Lieb et al., 2011; Lucas et al., 2011; Lu et al., 2014; Tian and Niu, 2015). An excessive input of N to soils often significantly changes soil exchangeable cations and their exchange capacity (Bowman et al., 2008; Gundersen et al., 2006; Lucas et al., 2011); high N input, for example, gradually depletes nutrient base cations (i.e., Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>, and Na<sup>+</sup>) and causes toxic metal ions (i.e., Al<sup>3+</sup>, Fe<sup>3+</sup>, and Mn<sup>2+</sup>) to accumulate in soils (Lucas et al., 2011). These N-induced negative effects on soil chemical processes are an important threat to ecosystem functioning, such as decreasing soil buffering capacity, decline of plant productivity (Stevens et al., 2010; Chen et al., 2013), and inhibition of soil biological processes (Kuperman and Edwards, 1997). In addition, N deposition also has a significant effect on soil microbial biomass and community structure (Frey et al., 2004; Waldrop et al., 2004). At global scale, a meta-analysis showed that anthropogenic N additions have been estimated to induce a reduction of soil microbial biomass by 15% (Treseder, 2008).

Our current understanding of how and the extent to which N deposition affects forest soil chemical properties and microbial communities, however, is largely derived from field experiments with understory addition of N (UAN; N is directly added to the forest floor). Apparently, forest canopy processes have been overlooked in most field N manipulation experiments. In fact, most N deposited from the atmosphere does not reach the forest floor directly but instead first contacts and passes through the canopy layer (Gaige et al., 2007; Wortman et al., 2012; Zhang et al., 2015). In this process, the forest canopy can retain a substantial proportion of the deposited N (Gaige et al., 2007) which could be taken up by canopy tree leaves and/or transformed into other forms (Adriaenssens et al., 2012), thus changing the quality and quantity of N deposited onto forest soils (Houle et al., 2015). A recent study suggested that, in failing to consider the effect of the forest canopy, previous N addition experiments may have overestimated the effects of N deposition on forest soils and related processes, and pose a great challenge for understanding the patterns and dynamics of forest ecosystems under N deposition in the future (Zhang et al., 2015).

However, no experimental studies have yet been performed to assess whether the effects of N deposition on forest soils differ depending on whether N is directly added to the forest floor or is added above the forest canopy.

In the present study, we conducted a field experiment that included both understory addition of N (UAN) and canopy addition of N (CAN) treatments in two contrasting forests (a temperate deciduous forest and a subtropical evergreen forest) in central and southern China (Zhang et al., 2015). The objective of this study was to determine if these two approaches of N addition (CAN vs. UAN) have different effects on forest soil abiotic (exchangeable cations) and biotic (microbial biomass) properties. As previous studies demonstrated, ecosystem background and the level of N addition often influence the responses of soil properties to N deposition (Treseder, 2008; Lu et al., 2011; Tian and Niu, 2015). Therefore, we also examine if forest type and N addition rate would alter the effects of N treatment approach (CAN vs. UAN). We hypothesized that the effects on both soil exchangeable cations and microbial biomass are weaker for CAN than for UAN, as predicted by Zhang et al. (2015). We also hypothesized that the effects of N treatment approach (CAN vs. UAN) would be affected by both forest type and N addition rate.

## 2. Materials and methods

### 2.1. Study sites

The experiment was conducted in two contrasting forest sites both located in climate transitional zones: the Jigongshan (JGS) site and the Shimentai (SMT) site. The JGS site was located in the Jigongshan National Nature Reserve (31°46′–31°52′ N, 114°01′–114°06′ E), Henan Province, Central China, which is in a climate transitional zone from subtropical to warm temperate. The mean annual temperature at the JGS Reserve is 15.2 °C, and the mean annual rainfall is 1119 mm. The background rate of N deposition in precipitation is about 19.6 kg N ha<sup>-1</sup> yr<sup>-1</sup> in this region (Zhang et al., 2015). The dominant vegetation at the JGS site was a deciduous temperate forest, which was 45 years old at the time of the study. The dominant canopy tree species at the JGS site were *Quercus acutissima* Carruth., *Quercus variabilis* Bl., and *Liquidambar formosana* Hance. The region has a yellow-brown sandy-loam soil (Zhang et al., 2015).

The SMT study site was in the Shimentai National Nature Reserve (24°22′–24°31′ N, 113°05′–113°31′ E), Guangdong Province, southern China, which is dominated by a subtropical monsoon climate with alternating wet and dry seasons. The mean annual temperature is 20.8 °C, and the mean annual rainfall is 1700 mm. The rate of N deposition in precipitation is about 34.1 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Zhang et al., 2015), with equal quantities of oxidized and reduced forms of N (Fang et al., 2011; Huang et al., 2012). The dominant vegetation at the SMT site was a broadleaved evergreen forest, which was 50 years old at the time of the study. The dominant canopy tree species at the SMT site were *Cryptocarya concinna*, *Schima superba*, *Machilus chinensis*, *Castanea henryi* (Skan) Rehd., and *Engelhardtia roxburghiana* (Zhang et al., 2015). The site has a latosolic red clay-loam soil (Zhang et al., 2015).

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