Environmental Pollution 235 (2018) 293-301

Contents lists available at ScienceDirect

### **Environmental Pollution**

journal homepage: www.elsevier.com/locate/envpol

# An increase in precipitation exacerbates negative effects of nitrogen deposition on soil cations and soil microbial communities in a temperate forest<sup> $\star$ </sup>

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#### ARTICLE INFO

Article history: Received 25 April 2017 Received in revised form 19 December 2017 Accepted 22 December 2017

Keywords: Canopy nitrogen deposition Precipitation increase Soil exchangeable cations Soil microbial communities Temperate forest

#### ABSTRACT

World soils are subjected to a number of anthropogenic global change factors. Although many previous studies contributed to understand how single global change factors affect soil properties, there have been few studies aimed at understanding how two naturally co-occurring global change drivers, nitrogen (N) deposition and increased precipitation, affect critical soil properties. In addition, most atmospheric N deposition and precipitation increase studies have been simulated by directly adding N solution or water to the forest floor, and thus largely neglect some key canopy processes in natural conditions. These previous studies, therefore, may not realistically simulate natural atmospheric N deposition and precipitation increase in forest ecosystems. In a field experiment, we used novel canopy applications to investigate the effects of N deposition, increased precipitation, and their combination on soil chemical properties and the microbial community in a temperate deciduous forest. We found that both soil chemistry and microorganisms were sensitive to these global change factors, especially when they were simultaneously applied. These effects were evident within 2 years of treatment initiation. Canopy N deposition immediately accelerated soil acidification, base cation depletion, and toxic metal accumulation. Although increased precipitation only promoted base cation leaching, this exacerbated the effects of N deposition. Increased precipitation decreased soil fungal biomass, possible due to wetting/re-drying stress or to the depletion of Na. When N deposition and increased precipitation occurred together, soil gram-negative bacteria decreased significantly, and the community structure of soil bacteria was altered. The reduction of gram-negative bacterial biomass was closely linked to the accumulation of the toxic metals Al and Fe. These results suggested that short-term responses in soil cations following N deposition and increased precipitation could change microbial biomass and community structure.

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#### 1. Introduction

Soils are central organizing entities in earth systems due to their vital roles in regulating carbon and nutrient cycling, decomposition, atmospheric compositions, water quality and plant productivity (Coleman et al., 2004). The world soils are currently being subjected to a number of anthropogenic global change factors (Bardgett and Wardle, 2010; Montanarella et al., 2016), including increases in N deposition and precipitation (Nielsen and Ball, 2015; Tian and Niu, 2015). Although, many studies have increased our understanding of how such global change factors affect soil







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chemical and biological properties (Blankinship et al., 2011; Pritchard, 2011; Tian and Niu, 2015), and soil functioning (Bardgett and van der Putten, 2014; Brady and Weil, 2016), most have focused on single factors or on the interactive effects of single factors with CO<sub>2</sub> increase and warming. It therefore remains unclear how N deposition and increased precipitation, two naturally co-occurring anthropogenic global change drivers, interactively affect soil chemical and biological properties.

Atmospheric N deposition has increased dramatically since the industrial revolution due to fertilization and fossil fuel burning (Galloway et al., 2008; Peñuelas et al., 2012), and increases in N inputs greatly affect soil chemical properties (Lucas et al., 2011; Tian and Niu, 2015). N deposition causes soil acidification (Lu et al., 2014; Tian and Niu, 2015; Mao et al., 2017), which has become a significant threat to soil functions (Kunhikrishnan et al., 2016). On the one hand, N-induced soil acidification can lead to losses of nutrient base cations (i.e., Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>, and Na<sup>+</sup>) (Tomlinson, 2003; Lucas et al., 2011; Shi et al., 2016); the loss of these cations not only decreases the availability of these nutrients (Lucas et al., 2011) but also reduces soil buffering capacity (Bowman et al., 2008; Lu et al., 2015). On the other hand, N-induced soil acidification can increase soil Al<sup>3+</sup> (Högberg et al., 2006), which can be directly toxic to soil organisms and plant roots (Piña and Cervantes, 1996; Poschenrieder et al., 2008).

N deposition also alters soil microbial dynamics (Waldrop et al., 2004; Ramirez et al., 2012) and reduces microbial biomass by an average of 15% (Treseder, 2008). The copiotrophic hypothesis suggests that N deposition may stimulate copiotrophic microbes (microbial taxa that have higher N demands and are restricted in catabolizing labile C pools), such as gram-negative bacteria, by alleviating the N limitation (Ramirez et al., 2012). However, the N-induced shifts in microbial community compositions are still unclear; some studies have indicated that N deposition decreases soil fungal biomass and fungal: bacterial ratios (Demoling et al., 2008), whereas other studies showed that fungal biomass did not change or even increased under N deposition (Gallo et al., 2004). It therefore remains unclear whether soil microbial community shifts under N deposition are predictable.

Apart from N deposition, precipitation is predicted to change across the globe (Stocker et al., 2013), with precipitation amounts being projected to increase in temperate regions of China (Yang et al., 2015). Previous studies showed that changes in precipitation regimes significantly altered the abiotic and biotic properties of soils (Austin et al., 2004; Nielsen and Ball, 2015). Because water availability is among the most important controls of soil chemical processes (Nielsen and Ball, 2015), increases in soil moisture resulting from increases in precipitation will substantially affect soil chemistry (Austin and Vitousek, 1998; Cregger et al., 2014). For example, several studies showed that increases in precipitation increased the rates of weathering, erosion and leaching and decreased the concentrations of rock-derived cation nutrients (Austin and Vitousek, 1998; Nielsen and Ball, 2015). Changes in precipitation regimes can also alter soil microbial growth and community composition (Cregger et al., 2012; Bell et al., 2014; Barnard et al., 2015). A number of studies have reported that soil microbial biomass increased in response to increased precipitation (Liu et al., 2009; Bell et al., 2014). Likewise, soil microbial community composition appears to be sensitive to changes in precipitation. The ratio of fungal-bacterial biomasses, for example, was increased by increased precipitation (Bi et al., 2012; Bell et al., 2014).

Although the effects of changes in N deposition and precipitation on soil chemistry and microorganisms have been separately studied (Treseder, 2008; Nielsen and Ball, 2015; Tian and Niu, 2015), these two factors are likely to interact but that interaction has seldom been assessed. This study therefore aimed to understand the separate and combined effects of N deposition and increased precipitation on soil exchangeable cations and soil microorganisms. We focused on soil exchangeable cations and soil microorganisms in this study for two reasons. First, both of these two soil properties are sensitive to environmental changes (Schimel et al., 2007: Lucas et al., 2011). Second, soil exchangeable cations (especially Al and Fe) can strongly affect soil microbial communities (Piña and Cervantes, 1996; Treseder, 2008). For this purpose, we conducted a novel field canopy N deposition and precipitation experiment with factorial design in a temperate deciduous forest. In contrast to most studies, which typically add N and water directly to the soil, the N and water in the current study were added to the canopy. This was done because recent studies have indicated that retention and other forest canopy processes are important in understanding the real effects of N deposition on soil properties (Gaige et al., 2007; Zhang et al., 2015). Based on the current knowledge about the effects of N deposition and increased precipitation on soil properties, we tested the following two hypotheses: (1) precipitation increase will exacerbate the negative effects of canopy N deposition on soil cations, i.e. enhancing losses of soil base cations; (2) precipitation increase will cancel out the negative effects of canopy N deposition on soil microbial biomass.

#### 2. Materials and methods

#### 2.1. Study site

The experiment was conducted at the ligongshan (IGS) National Nature Reserve (31°46'-31°52' N, 114°01'-114°06' E) in Henan Province, Central China. The reserve is located in a transitional zone between a subtropical and a warm temperate climate. The mean annual precipitation is 1119 mm and the mean temperature is 15.2 °C (Zhang et al., 2015). During the study, the annual precipitation is 971 mm and 1150 mm in year 2013 and year 2014 respectively. 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 reserve is evenly covered by a mixed deciduous forest. The forest at the study site is comprised of several canopy tree species, including Quercus acutissima Carruth., Quercus variabilis Bl., and Liquidambar formosana Hance. The soil in the reserve is Cambisols based on FAO soil classification system (IUSS Working Group WRB., 2015). The soil texture is sandy loam. The organic carbon content of the surface soil (0-10 cm) is  $60 \text{ mg g}^{-1}$  dry soil, and the soil clay minerals are mainly kaolinite and illite ilerite (Shi, 2016).

#### 2.2. Experimental design

Using a completely randomized block design, we established 16 circular experimental plots (907  $\text{m}^2$  per plot; four blocks × four treatments) in the summer of 2012 at the JGS forest. Within each of the four blocks, each of the four plots was assigned one of four treatments: control (CK), canopy N addition (CN), canopy water addition (CW), and  $CN \times CW$  (CNW). The canopy addition of N and water was designed to realistically simulate atmospheric N deposition and precipitation in forests (Houle et al., 2015; Zhang et al., 2015). The CN treatment received 50 kg N ha<sup>-1</sup> yr<sup>-1</sup>, a rate that is projected to occur in the near future in this region (Liu et al., 2013). Considering the background N deposition rate, the exact N deposition in this study above the forest canopy is 70 kg N ha<sup>-1</sup> yr<sup>-1</sup>. N was applied as an aqueous solution of NH<sub>4</sub>NO<sub>3</sub> at monthly intervals during the growing season (April to October). NH<sub>4</sub>NO<sub>3</sub> (the ratio of NH<sub>4</sub>-N to NO<sub>3</sub>-N is 1) used in study because the ratio of NH<sub>4</sub>-N to NO<sub>3</sub>-N (2) in current deposition was predicted to decrease in future due to the more rapid percentage increase in NO<sub>x</sub> emissions than in

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