

**Nitrogen saturation and light availability influence the growth of
understory plants in a nitrogen-fertilized subtropical forest: Reply to Taiki
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As atmospheric nitrogen (N) deposition has been a noteworthy aspect of global change, N fertilization experiments using ammonium nitrate (NH_4NO_3) are commonly applied to simulate the potential effects of N enrichment on growth of plants (e.g. Högberg et al., 2006; Lu et al., 2010; Alvarez-Clare et al., 2013; Minocha et al., 2015) and functioning of forest ecosystems (e.g. Magill et al., 2004; Cleveland and Townsend, 2006; Mo et al., 2008; Gurmesa et al., 2016). Taking the increasing N deposition in eastern China into consideration (Liu et al., 2013), we set the dosages of N fertilization as $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (abbreviated as N50) and $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (N100) to examine potential effects of high N deposition on growth of trees and understory plants in subtropical forest ecosystems (Tian et al., 2017a). The dosages of N50 and N100 were also used in many of previous studies conducted in boreal and temperate forests in Europe and America, and tropical and subtropical forests (e.g. Rainey et al., 1999; Högberg et al., 2006; Lu et al., 2010; Alvarez-Clare et al., 2013).

After 3.4 years' N fertilization, we found significant decreases in growth rates of small trees, understory saplings, shrubs, seedlings and ferns, and insignificant changes in those of large trees with $\text{DBH} > 10 \text{ cm}$. We proposed several possible mechanisms that regulate these different responses for trees and understory plants, such as nutrient limitation, low understory

light availability, and potential N saturation (Tian et al., 2017a). Recently, Mori (2017) commented our paper and questioned some of our explanations. Mori's comments can be summarized as two points: (1) we didn't consider the impact of 'fertilizer burn'; and (2) he cast doubt on our viewpoint about the negative effects of understory light limitation and potential N saturation.

We appreciate his comments, but wonder if he over-stated the direct impact of NH_4NO_3 solution on plant leaves in our experiment and ignored differences of the NH_4NO_3 application between forest and agricultural ecosystems. We agree that the "fertilizer burn" is common to crops with extremely high concentration of N on leaves in agricultural ecosystems (Neumann et al., 1981; Fageria et al., 2009). However, the possibility of direct exposure of leaves to NH_4NO_3 solution and the risk of direct foliar fertilizer damage on the understory in our experiment are very low, because our experiment was designed to explore the effects of enhanced N entering soil on plant growth and ecological functions in the subtropical forest ecosystem, in which direct leaf fertilization must be avoided to eliminate the risk of potential foliar damage.

In our experiment, the NH_4NO_3 solution was applied to the ground carefully by a back-hatch sprayer, but not sprayed directly onto leaves of plants (Photo 1), although spraying may exert some effects on leaves of some ground-cover plants (such as *Woodwardia japonica*). Moreover, annual precipitation and throughfall in this humid subtropical forest are quite high (1700 and 1500 mm, respectively), which scour the plant leaves frequently and thus wipe out the risk of leaf NH_4NO_3 solution residual even when the ground-cover ferns are accidentally exposed to the NH_4NO_3 solution. As a direct evidence of negligible effects of spraying NH_4NO_3 solution on the leaves, Photo 2 shows clearly no significant difference in plant leaf growth performances before and after NH_4NO_3 solution application. Hence, the direct 'foliar fertilizer damage' raised by Mori (2007) could be hard to explain the negative effects of N fertilization on understory plants in our experiment.

Why N fertilization decreased the growth of understory plants? Light availability and soil nutrient condition were regarded as two main factors shaping growth of understory plants (e.g. Rainey et al., 1999; Dirnböck et al., 2014; Gurmesa et al., 2016; Walter et al., 2016). Accordingly, we highlighted the effects of these two factors on the growth of understory plants in our paper (Tian et al., 2017a). Here, again we would like to state some more about these two factors.



Photographed by Di Tian (2015/07/10)

Photo 1. The application of NH_4NO_3 solution on the ground using a back-hatch sprayer during our experimental process.



Photo 2. Understory plants before and after NH_4NO_3 solution application, which were taken in July 2015 to illustrate if there are differences in leaf growth performance before and after the N application. (a) - (b) represent *Sarcandra glabra*; (c) - (d) represent *Cleyera japonica*; and (e) - (f) represent *Camellia cuspidata*.

As light availability plays a critical role in nutrient utilization and photosynthesis of understory plants in the closed forests (Strengbom and Nordin, 2008; Alvarez-Clare et al., 2013; Record et al., 2016), we took a series of canopy photos in different N treatments to illustrate if there are differences in leaf morphology and tree canopy cover among N treatments. Although the data of canopy cover estimated by weighted ellipsoidal method showed no remarkable differences, which might be resulted from the fluctuation of understory light irradiance during a day, we found tighter and denser crown of trees in the N fertilized plots during our field observation. Given the denser tree canopy in the N fertilized plots, we inferred the possibility of self- and alien thinning of individuals, especially of the small-sized plants in those plots, because of their light competition. This inference resonates with the views of previous studies that trees tended to allocate more carbon and nutrient to aboveground organs to stimulate the expansion of tree crown to gain more light resources with nutrient fertilization, which may result in reduction of light availability and difficulty of nutrient interception for understory plants (Alvarez-Clare et al., 2013; Schroth et al., 2015; Ibáñez et al., 2016).

The N saturation is suggested as another major factor influencing growth of understory plants in our paper (Tian et al., 2017a). Many previous studies have revealed that N saturation after long-term N fertilization influenced significantly plant growth and forest ecosystem functioning (e.g. Aber et al., 1998; Wallace et al., 2007; De Schrijver et al., 2007), because excess N accumulation beyond the demand of ecosystems may induce changes in soil environment (e.g. bulk density, pH, nutrient content, microbial community) and plant nutrient stoichiometric characteristics (DeHayes et al., 1999; Höglberg et al., 2006; Mayor et al., 2014). For example, N application at Harvard Forest showed that N addition increased the partitioning of excess N into foliar stress-related metabolites, resulting in the higher mortality of pines (Minerva et al., 2015). With excessive N accumulated in soil, aggregated soil acidification altered the balance of base cation and micronutrient (Wang et al., 2017) and microbial community (Geisseler and Scow, 2014). In particular, the mobilization and accumulation of phytotoxic metal ions, such as aluminum (Al^{3+}) and manganese (Mn^{2+}), could suppress photosynthetic capability and survival of plants (Lu et al., 2014). Hence, plant growth in the subtropical forest in our study could be influenced by the potential N saturation that was inferred from the decreasing soil pH, increasing soil N and Mn concentrations, disturbed microbial community, and reduction of understory plant biomass following the N fertilizations (Tian et al., 2017a and 2017b). That there were no significant correlations between soil total N content and understory biomass, argued by Mori (2017), may be partly

because of the confounding effects of soil nutrient heterogeneities and low plot replications. Similar viewpoint was also claimed in a nutrient-fertilized tropical forest (Alvarez-Clare et al., 2013).

In conclusion, Mori's criticisms on our explanations for the lower understory growth under N addition are incorrect. Considering that some uncertainties remain in our study, we take this opportunity to suggest that further observations are required to explore the mechanisms underlying the changes of different growth forms with the effects of N addition in the subtropical forests. We also suggest that more attention should be paid to the plant-soil interactions to untangle the confounding effects of N fertilization and other factors on plant growth in this subtropical forest as shown in our previous studies (Tian et al., 2017a and 2017b).

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