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Effects of simulated nitrogen deposition increase on plant nutritional status and physiological responses at two contrasting Beech forest sites in Italy

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Anthropogenic activities resulted in a significant increase in nitrogen (N) compounds in the atmosphere and their deposition back to the biosphere, with important implications on both carbon (C) and N cycles. Indeed, an increase in N deposition can increase forest productivity in N limited forest ecosystems. In addition, it can also increase N loss pathways, leading to soil acidification and nutrient imbalance. Several N manipulation experiments have been carried out for decades till now, though most of them focused on conifer forests. We consider two manipulation experiments established in 2015 on two beech (*Fagus sylvatica* L.) forests in Italy, Cansiglio and Collelongo sites, located on the Eastern Alps and Central Apennines, respectively. The two forests were chosen along a climate and N deposition gradient. Thus, our goal was to assess the effects of simulated N deposition increase on nutritional, physiological status and growth of beech forests from two contrasting climatic conditions. At both sites, N was added directly to the soil as NH_4NO_3 in two doses: $30 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ and $60 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Moreover, in Cansiglio we also included a canopy N fertilization adding $30 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Leaves were collected in 2016 and 2018 for the analyses of nutrients, stable C and N isotopes, and photosynthetic pigments. The aboveground production was periodically monitored with girth band and litterfall collectors. The nutrient stoichiometry analysis showed elevated N concentrations and high N:P in both forests, even in the control plots. N addition significantly increased N:P and N:S ratios in the treated plots. Changes in chlorophyll concentration were mainly related to differences between the two sites, while carotenoids were also influenced by N fertilization. After four years, we did not find an effect of the treatment (regardless of the doses) on both tree growth and leaf biomass. Altogether, our results suggest that both forests were not N limited. Finally, difference between the two manipulation approaches will be discussed in terms of leaf nutrients and C and N stable isotopes in the case of Cansiglio site.

