The stable isotopic compositions of nitrate extracted from natural plants : Quantifying the contribution of atmospheric nitrogen oxides to nitrogen assimilated by plants

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Nitrogen is one of the essential elements for organisms in general. Nitrate is the representive nitrogen nutrient assimilated by plants. While most of nitrate assimilated by plants must be supplied from soils, plants could uptake atmospheric nitrogen oxides from leaves and use them for the source of nitrogen. Traditionally, such assimilation pathways in plants of have been studied using artificial isotope tracers such as ^{15}N. The ^{15}N tracer methods, however, need tedious and time consuming incubation expariments. Besides, such experiments could often alter the growing environment from natural. In order to clarify both the source and the assimilation pathway of nitrate in plants, we used the natural stable isotopes of nitrate extracted from plants as tracers, instead of artificialy concentrated isotopes, with special emphasis on quantifying the contribution of atmospheric nitrogen oxides to plants by measuring triple oxygen isotopic compositions of nitrate.
Leaf samples were collected from conifer needles (*Metasequoia* and *Cypress*), broad leaves (*Quercus glauca* and *Prunus yedoensis*) and herbage (*Sasa*) once every month from April, 2016, to April 2017 from the same plant individual at Nagoya University.The leaf samples were washed immediately after each sampling using Milli-Q water and dried, and then nitrate was extracted using Milli-Q water, while insoluble materials were removed through filiteration.The stable isotopic compositions including the triple oxygen isotopic compositions of the extracted nitrate were determined using Continuous-Flow Isotope Ratio Mass Spectrometry (CF-IRMS) system in Nagoya University (Tsunogai et al., 2010).
The nitrate concentrations per dry weight of plants were ranged from 0.1 to 0.5 μmol g^{-1}. We could not find clear seasonal variations in the concentrations. The isotopic compositions of nitrate extracted from the plants were highly deviated from those in soil water. The $\delta^{15}N$ values showed large variation from -25‰ to +10‰ (vs. air) among the plants. *Quercus glauca* and *Metasequoia* showed definitely lower $\delta^{15}N$ values in nitrate compared with those dissolved in soil water (-5‰ to +5‰). Besides, the $\delta^{18}O$ values were significantly higher than those dissolved in soil water (-10‰ to 0‰ vs. VSMOW) , ranging from +60‰ to +80‰ in the whole plants. Furthermore, the $\Delta^{17}O$ values always showed large anomalies (+13‰ to +20‰). We concluded that most of the nitrate extracted by the method contain nitrate derived from atmospheric nitrogen oxides.

Keywords: Plant, Nitrogen oxides, Triple oxygen isotopic composition

