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Nitrate is an important nitrogen source for Arctic tundra plants

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Plant nitrogen (N) use is a key component of the N cycle in terrestrial ecosystems. The supply of N to plants affects community species composition and ecosystem processes such as photosynthesis and carbon (C) accumulation. However, the availabilities and relative importance of different N forms to plants are not well understood. While nitrate (NO−3) is a major N form used by plants worldwide, it is discounted as a N source for Arctic tundra plants because of extremely low NO−3 concentrations in Arctic tundra soils, undetectable soil nitrification, and plant-tissue NO−3 that is typically below detection limits. Here we reexamine NO−3 use by tundra plants using a sensitive denitrifier method to analyze plant-tissue NO−3. Soil-derived NO−3 was detected in tundra plant tissues, and tundra plants took up soil NO−3 at comparable rates to plants from relatively NO−3-rich ecosystems in other biomes. Nitrate assimilation determined by 15N enrichments of leaf NO−3 relative to soil NO−3 accounted for 4 to 52% (as estimated by a Bayesian isotope-mixing model) of species-specific total leaf N of Alaskan tundra plants. Our finding that in situ soil NO−3 availability for tundra plants is high has important implications for Arctic ecosystems, not only in determining species compositions, but also in determining the loss of N from soils via leaching and denitrification. Plant N uptake and soil N losses can strongly influence C uptake and accumulation in tundra soils. Accordingly, this evidence of NO−3 availability in tundra soils is crucial for predicting C storage in tundra.

Nitrogen (N) is often the nutrient that most limits terrestrial plant growth, making plant N availability a key determinant of primary productivity in terrestrial ecosystems (1). Hence, improved knowledge of in situ plant N availability and consequent plant N use is crucial for better evaluating and predicting responses of vegetation to climate change and N loading (2, 3). However, the availability of N to terrestrial plants is difficult to evaluate using measurements of soil N because of strong plant–microbe and plant–plant competition for N and the resulting rapid turnover of soil N pools (4).

Arctic ecosystems are typically characterized by strong N limitation (1). Because of high carbon (C) stocks in permafrost soil and their sensitivity to environmental change, the Arctic C cycle has important implications for global C balance and C-climate feedbacks (5, 6). Although it remains difficult to budget N inputs in the Arctic, the Arctic biome is a potential sink for anthropogenic N pollutants (7). So far, long-term N addition experiments have revealed that elevated N inputs into Arctic tundra ecosystems change C accumulation and species diversity (5, 8, 9). Field observations and isotope labeling experiments provide evidence of how added N has altered the distribution, fate, biotic use, and losses of N in Arctic tundra ecosystems (10–15). These studies indicate that a better understanding of in situ N availability in Arctic ecosystems is important because C and N cycles are tightly coupled between the vegetation and soils, and elevated N loading can influence the Arctic’s C balance (5, 16).

Nitrate (NO−3) is a common and pivotal plant-available N form in addition to ammonium (NH4+) and some forms of dissolved organic N (DON) (1). Until the 1990s, researchers underestimated the availability of soil NO−3 to microbes because microbial uptake of NO−3 often results in very low NO−3 standing stock and low or negative net NO−3 production (nitrification) rates in soil, even when gross nitrification rates are high (17–19). However, it remains undetermined how important soil NO−3 is for plants because of inadequate understanding of in situ plant NO−3 use. In Arctic tundra, NO−3 availability can be increased by direct release from thawing permafrost, melting snow, and increased nitrification resulting from elevated N loading and warming

Significance

How terrestrial plants use N and respond to soil N loading is central to evaluating and predicting changing ecosystem structure and function with climate warming and N pollution. Here, evidence from NO−3 in plant tissues has uncovered the uptake and assimilation of soil NO−3 by Arctic tundra plants, which has long been assumed negligible. Soil NO−3 contributed about one-third of the bulk N used by tundra plants of northern Alaska. Accordingly, the importance of soil NO−3 for tundra plants should be considered in future studies on N and C cycling in Arctic ecosystems where C sequestration is strongly determined by N availability.

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temperatures (7, 14, 20). Elevated NO$_3^-$ availability to tundra plants can change interspecific N competition and N-use strategies of tundra plants (9, 13, 21), potentially resulting in the spread of NO$_3^-$-adapted species and altering the partitioning of aboveground vs. below-ground biomass (18, 22–24). These factors could alter CO$_2$ fixation by vegetation and the quantity and quality of litter inputs to the soil, which would then change microbial breakdown of soil C and the emission and uptake of greenhouse gases (5, 8, 25–27). Accordingly, soil NO$_3^-$ availability and plant NO$_3^-$ use have important implications for both N and C cycles in Arctic tundra.

Despite its potential importance, NO$_3^-$ availability and the contribution of different N forms to plant N use have been unclear in Arctic tundra (21, 28). Four decades of research show that tundra plants rely on soil NH$_4^+$ and DON (e.g., direct uptake of free amino acids) to meet growth requirements for N (12, 21, 28–31). In contrast, researchers generally have considered plant NO$_3^-$ use to be negligible in the Arctic for several reasons. First, NO$_3^-$ concentrations in soils are often low or undetectable, and soil net nitrification rates seldom show positive values (SI Appendix, Figs. S1 and S2), presumably because of low temperature, low soil NH$_4^+$ availability, and low soil pH, together with high microbial N demand (32, 33). Second, plant-tissue NO$_3^-$, a common marker of plant NO$_3^-$ uptake, is rarely detected in tundra plants with conventional analytical methods (11, 12, 34). We argue that the importance of NO$_3^-$ to plants in such seemingly low-NO$_3^-$ Arctic tundra ecosystems remains an open question for several reasons. First, although extractable soil NO$_3^-$ concentrations are typically low in Arctic tundra soils, NO$_3^-$ is sometimes present in measurable amounts and contributes non-trivial fractions of total extractable N (TEN) stocks similar to high-NO$_3^-$ ecosystems (SI Appendix, Fig. S2B). Second, rates of in situ NO$_3^-$ reductase activity (NRA), which is inducible and re-detected in plants of this study. Besides, while NO$_3^-$ availability and plant NO$_3^-$ use have important implications for both N and C cycles in Arctic tundra.

**Results and Discussion**

Using the highly sensitive denitrifier method (detailed in Materials and Methods), we analyzed concentrations and stable isotope compositions of NO$_3^-$ in tissues of dominant plant species in Alaskan tundra ecosystems. We then compared our results with those for plants from relatively high-N or high-NO$_3^-$ ecosystems in lower-latitude regions (Figs. 1 and 2). Such comparisons of Arctic sites to non-Arctic sites, using both traditional and new methods, are important for understanding soil N cycling (particularly soil NO$_3^-$ availability) and for placing the N uptake abilities of tundra plants into a broader context.

**The Uptake of NO$_3^-$ in Plants.** The existence of NO$_3^-$ in plant tissues is evidence for NO$_3^-$ uptake from the soil or atmosphere because NO$_3^-$ production in non-N$_2$-fixing plants is negligible under normal conditions (36–40). Although NO$_3^-$ can be produced from the oxidation of nitric oxide (NO) both enzymatically and non-enzymatically in non-N$_2$-fixing plants (37–40), the rates are very low in natural environments (41–44), especially compared with the pool sizes of NO$_3^-$ detected in plants of this study. Besides, while NO$_3^-$ production by nonsymbiotic hemoglobin is possible in anoxic conditions (38, 39) and with high ambient NO concentrations (40), neither anoxic conditions nor high ambient NO applies to the present study. We detected unexpectedly high NO$_3^-$ concentrations in leaves and roots of the tundra plant species studied (Fig. 1 and SI Appendix, Tables S1 and S2). First, of the 153 tundra plant samples analyzed, 143 had measurable NO$_3^-$ concentrations (detailed in Materials and Methods). Some species (e.g., Polygonum bistorta) had higher foliar NO$_3^-$ than low-latitude forest species, including those in high-NO$_3^-$ environments (Fig. L4 and SI Appendix, Table S2). Second, ratios of leaf NO$_3^-$ to soil NO$_3^-$ and of root NO$_3^-$ to soil NO$_3^-$ were similar between tundra and lower-latitude ecosystems or even higher in tundra than in some lower-latitude ecosystems (SI Appendix, Fig. S4). These results provide evidence of high NO$_3^-$ uptake of tundra plants despite much lower concentrations of NO$_3^-$ in tundra soils. Thus, we conclude that tundra plants can take up NO$_3^-$ as efficiently as plants from relatively NO$_3^-$-rich ecosystems in other biomes. In addition, NO$_3^-$ additions to soils enhanced leaf NO$_3^-$ concentrations in most tundra plants (SI Appendix, Figs. S5 and S6). This result is evidence that plant NO$_3^-$ uptake is responsive to soil NO$_3^-$ variations in Arctic tundra ecosystems. Such responses and patterns of NO$_3^-$ uptake among studied species are useful for interpreting
changes in functional traits and the structure of tundra plant communities in response to projected increases of soil NO$_3^-$ with climate warming and elevated N deposition (1, 45).

**The Sources of NO$_3^-$ in Plants.** We used the $\Delta^{17}$O signatures of leaf NO$_3^-$ ($^{17}$O$_{\text{leaf}}$) to verify the mixing of atmospheric-derived NO$_3^-$ ([$^{17}$O$_{\text{atm}}$] > 0 per mille ($‰$)) due to an enrichment in $^{17}$O during photochemical oxidation of nitrogen oxides (NOx) by O$_3$ with soil-derived NO$_3^-$ ($^{17}$O$_{\text{soil}}$ = 0‰ because of no $^{17}$O excess in atmospheric O$_3$ and soil H$_2$O molecules) (46–48). Leaf NO$_3^-$ of *P. bistorta* showed no $^{17}$O isotope anomaly ($^{17}$O$_{\text{values}}$ = 0.0%e; *SI Appendix*, Fig. S7), indicating that the NO$_3^-$ detected in this species was purely soil derived. Clearly, soil NO$_3^-$ is available to, and taken up by, tundra plants.

In contrast, positive $^{17}$O$_{\text{leaf}}$ values in low-latitude forests (*SI Appendix*, Fig. S7) indicate the direct leaf absorption of atmospheric-derived NO$_3^-$ ($^{17}$O$_{\text{leaf}}$ > 0‰) or possibly the root uptake of NO$_3^-$ at the surface soil with positive $^{17}$O values (49). We used mean $^{17}$O values of precipitation NO$_3^-$ measured in the Tama-Kyuro Field Museum forest in temperate Japan (TML) (see *SI Appendix*, Table S1 for descriptions of the forest sites used in this study) (49); in Guiyang in subtropical China (this study); and in Jianfengling forests in Hainan, tropical China (49) as $^{17}$O$_{\text{atm}}$ values in the studied temperate, subtropical, and tropical forests, respectively (*SI Appendix*, Fig. S7). We then estimated mixing ratios of atmospheric-derived NO$_3^-$ ([$^{17}$O$_{\text{leaf}}$–$^{17}$O$_{\text{atm}}$]) for plants in lower-latitude ecosystems. The results showed that atmospheric-derived NO$_3^-$ accounted for, on average, 35% (6 to 86%) of total leaf NO$_3^-$ in measured samples from lower-latitude forests.

**NO$_3^-$ Assimilation in Plants.** Higher $^{15}$N and $^{17}$O values in plant-tissue NO$_3^-$ relative to source NO$_3^-$ could provide new evidence for in situ plant NO$_3^-$ assimilation because NO$_3^-$ reduction via NO$_3^-$ reductase would cause $^{15}$N and $^{17}$O enrichments in the unassimilated NO$_3^-$ (2, 50–52). Accordingly, we calculated differences ($\Delta$ values) between isotopic values of tissue NO$_3^-$ ($^{15}$N and $^{17}$O) in each plant sample and mean values of soil NO$_3^-$ in corresponding ecosystems (Fig. 2 and *SI Appendix*, Fig. S8).

In northern Alaska, $^{15}$N values of soil NO$_3^-$ were 1.0‰ at Toolik Field Station (TFS) (see *SI Appendix*, Table S2) and 0.5 ± 4.7‰ at Barrow (54). Atmospheric-derived NO$_3^-$ in snowmelt had lower $^{15}$N values of −4.8 ± 1.0‰ at Barrow (54) and much lower values of −8.6 ± 0.7‰ at a high Arctic site at Midtre Lovénbreen, Svalbard (55). Compared with $^{15}$N values of soil- or atmospheric-derived NO$_3^-$ (*SI Appendix*, Fig. S8A), the higher $^{15}$N values of leaf NO$_3^-$ in tundra of northern Alaska (positive $^{15}$N values; Fig. 2A) are evidence for in situ NO$_3^-$ assimilation in tundra plants.

In non-Arctic sites, higher $^{18}$O values of leaf NO$_3^-$ than those of soil NO$_3^-$ (positive $^{18}$O values; Fig. 2B) also provide evidence for in situ NO$_3^-$ assimilation in tundra plants.

We then estimated mixing lines of subtropical sites (*SI Appendix*, Fig. S7) and tropical sites (*SI Appendix*, Fig. S8) for in situ NO$_3^-$ assimilation in tundra plants. However, higher $^{18}$O enrichments (*SI Appendix*, Fig. S8) might be due, in part, to contributions from high $^{18}$O values of atmospheric-derived NO$_3^-$ (57). Major uncertainties existed in fractional contributions of atmospheric-derived NO$_3^-$ in leaf NO$_3^-$ because of limited $\Delta^{17}$O data of leaf NO$_3^-$ and lack of explicit $\Delta^{17}$O values of atmospheric NO$_3^-$.

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Fig. 2. Differences ($\Delta$ values) in $^{15}$N (A) and $^{17}$O (B) between leaf NO$_3^-$ and soil NO$_3^-$ across different ecosystems. The box encompasses the 25th to 75th percentiles, whiskers are the SD values, and the red line and red square in each box mark the median and mean values, respectively. Unique letters above the boxes indicate significant differences at the level of $P < 0.05$. The $\Delta$ values were calculated using replicate values of plant tissues minus mean values of soil in corresponding sites (*SI Appendix*, Fig. S8 and Table S1).

Fig. 3. $\Delta^{17}$O vs. $^{18}$O plots of NO$_3^-$ in soil, leaves, and atmospheric (Atmos, as precipitation or snow) deposition across different ecosystems. The mixing lines of Arctic and tropical sites ($y = 2.52x – 4.42$ and $y = 2.97x + 0.58$, respectively) were based on isotopic values of soil NO$_3^-$ ($n = 18$) (54) and snowpack NO$_3^-$ ($n = 12$) (56) at Barrow, and of soil NO$_3^-$ ($n = 18$) and precipitation NO$_3^-$ ($n = 3$) at Jianfengling in tropical China (49), respectively. The mixing line of temperate sites ($y = 2.64x + 3.82$) was based on isotopic values of soil NO$_3^-$ at Japanese temperate sites ($n = 22$) and precipitation at TML ($n = 12$) in this study. The mixing line of subtropical sites ($y = 2.87x + 0.91$) was based on isotopic values of soil NO$_3^-$ ($n = 29$) at subtropical sites and precipitation NO$_3^-$ at Guiyang, China ($n = 3$) in this study. The $\Delta^{17}$O of soil NO$_3^-$ was assumed to be zero.
Precipitation NO$_3^-$ might not fully represent all atmospheric NO$_3^-$ contributions to plant leaves; in addition, it is even more difficult to determine reasonable $\delta^{15}$N and $\delta^{18}$O end-member values of atmospheric-derived NO$_3^-$ in plant leaves. Despite these problems, NO$_3^-$ isotope values in plant tissues did provide information on plant NO$_3^-$ sources and uptake in disturbed ecosystems.

**Contributions of Soil NO$_3^-$ to Total N in Tundra Plants.** Compared with plants in relatively N-rich ecosystems, tundra plants showed a similar distribution of leaf total N concentrations but a much wider distribution of leaf total (bulk) $\delta^{15}$N values (SI Appendix, Fig. S9). The wider distribution of leaf total $\delta^{15}$N values arises because of the strong niche differentiation of N-use regimes among tundra plants (13, 38). However, $\delta^{15}$N values of total N in tundra plants (−11.2 to 5.3‰ in Alaska) are generally lower than those of soil NH$_4^+$ (around 12.3 ± 3.6‰) (this study); 4.4 ± 0.9‰ (53); and 1.4 ± 0.5‰ (21)), although some DON components are $^{15}$N depleted (around −5.7‰ for hydrolyzable amino acids (HAA) at Tsimaná Creek (IMT) in northern Alaska; see SI Appendix, Table S1) (Fig. 4). This disparity between the $\delta^{15}$N signatures of plant total N vs. soil N sources exists even when isotopic fractionations for NH$_4^+$ and HAA assimilation by mycorrhizal plants are considered. Given plant NO$_3^-$ uptake and assimilation as indicated by NO$_3^-$ in plant tissues, soil NO$_3^-$ should be considered when using $\delta^{15}$N methods to evaluate in situ contributions of soil N sources to total N of tundra plants.

Proportional contributions ($f$, expressed as a percentage) of soil NO$_3^-$ to total N in tundra plants were estimated using $\delta^{15}$N values of soil N (NO$_3^-$, NH$_4^+$, and HAA) and $\delta^{15}$N values of leaf total N in a Bayesian isotope-mixing model [Stable Isotope Analysis in R (SIAR) (cran.r-project.org/web/packages/siar/index.html) (59)] (Fig. 5). The SIAR model uses a Bayesian framework to establish a logical prior distribution (60) for estimating $f$ values, and then determines the probability distribution for the $f$ values of each source (soil NO$_3^-$, NH$_4^+$, and HAA, in this study) to the mixture (total N of plant leaves, in this study). We contend that this approach provides reliable estimations of fractional contributions of different N sources to plant total N because the mixing model considers isotope effects during plant N uptake ($\delta^{15}$N values hereafter) and variability in both source $\delta^{15}$N values and plant $\delta^{15}$N values (61).

In this study, the $\delta^{15}$N values (mean ± SD) of soil NO$_3^-$ at Barrow [0.5 ± 4.7‰ (58)], soil NH$_4^+$ at IMT and TFS (11.5 ± 8.4‰, this study and ref. 21), and soil HAA at IMT [−5.7 ± 22.2‰; (53)] were used as source $\delta^{15}$N values. For nonmyncorrhizal (NM) plants, leaf $\delta^{15}$N values were mainly controlled by the $\delta^{15}$N values and $f$ values of source N (NO$_3^-$, NH$_4^+$, and HAA), assuming negligible isotope effects during the acquisition processes of source N from soil into NM plants (i.e., $\delta^{15}$N values were cited from ref. 54. For mycorrhizal plants, the $\delta^{15}$N values during the acquisition processes of soil N sources were calculated as the net differences of leaf $\delta^{15}$N values between mycorrhizal and NM plants. The same $\delta^{15}$N value was assumed for plant species associated with the same type of mycorrhiza and for N forms absorbed through the same type of mycorrhiza. In Alaskan tundra, the $\delta^{15}$N values for plant species associated with arbuscular mycorrhizae (AM), ectomycorrhizae (ECM), and ericoid mycorrhizae (ERM) were estimated as net $\delta^{15}$N differences from NM plants—that is, −5.0‰, −6.9‰, and −7.7‰, respectively (21, 62), which differed from the $\delta^{15}$N values normalized for worldwide plants [−2.0‰, −3.2‰, and −5.9‰, respectively (63)]. Our $\delta^{15}$N values (0‰ for NM plants, −5.0‰ for AM plants, −6.9‰ for ECM plants, and −7.7‰ for ERM plants)
were considered under four scenarios (scenario 1: for NO$_3^-$, NH$_4^+$, and HAA; scenario 2: for NH$_4^+$ and HAA only; scenario 3: for HAA only; and scenario 4: for none of NO$_3^-$, NH$_4^+$, and HAA) (Fig. 5). Estimates from natural $^15$N evidence were that NO$_3^-$ assimilated accounted for 4 to 52% of specific-leaf total N (around one-third, on average) of Alaskan tundra plants (Fig. 5), thereby demonstrating the importance of soil NO$_3^-$ relative to soil NH$_4^+$ and HAA for N use by many tundra plants. These findings also enhance understanding of N competition among plant species and between plants and microbes in Arctic tundra ecosystems, and how that may affect changes in species community composition and productivity with climate change and N pollution.

Materials and Methods

Study Sites and Sampling. To evaluate in situ NO$_3^-$ uptake and assimilation in terrestrial plants in relation to NO$_3^-$ availability, we selected 18 sites (see descriptions in SI Appendix, Table S1) across a distinct gradient of soil NO$_3^-$ (SI Appendix, Fig. S2), including one tropical and four subtropical sites in southwestern China; nine temperate sites in central, southern, and western Japan; and four Arctic tundra sites in northern Alaska. Among them, Tsukuba Forest Experimental Watershed (TKB) and Tama-Kyuyro Field Museum upper slope (TKB) and low slope (TMU) (SI Appendix, Table S1) are characterized by high soil NO$_3^-$ or N saturation (49, 64, 63), while the Arctic sites TFS, Sagavanirktok River Valley (SAG), and IMT (SI Appendix, Table S1) are characterized by unmeasurable nitrification rates and negligible soil NO$_3^-$ and, thus, are assumed to be typically low-NO$_3^-$ ecosystems (SI Appendix, Fig. S2). In total, 28 plant species in the above study sites were sampled for fine-roots (leaf mass, 1.0 mm in diameter and 20 cm in spatial distribution of soil depth) or mature sunlit leaves. The studied plants in each ecosystem include dominant indigenous species (SI Appendix, Table S1). The design of this study allows us to evaluate plant NO$_3^-$ use at the species and ecosystem levels.

Soil N Analyses. Soil N concentrations and N transformation rates (mineralization and nitrification) were measured as indices of potentially available NO$_3^-$ for both plants and soil microbes. Information on soil types and samplings, N variables, and corresponding methods used for each ecosystem are summarized in SI Appendix, Table S1. Concentrations of NO$_3^-$ and NH$_4^+$ in soil solutions, extracts of fresh soils, and extracts of incubated soils (for net N mineralization and net nitrification rates) were determined colorimetrically. TEN was digested to NO$_3^-$ using alkaline persulfate digestion and its concentration measured as NO$_3^-$ on the autoanalyzer (specified in SI Appendix, Table S1). In-house standards (alanine, glycine, and histidine) dissolved in corresponding extracts were used for calibrating the concentrations of TEN and estimating the effect of the N blank from reagents (the same as that described in ref. 65). The soil extractable organic N was calculated as the difference between soil TEN and extractable inorganic N. $^{15}$N and $^{13}$C ratios of soil NO$_3^-$ were determined using the denitrifier (Pseudomonas auresfaciens) method (described in refs. 65 and 66) that converts NO$_3^-$ to nitrous oxide (N$_2$O) (67, 68). The calibration curve between measured isotope ratios of N$_2$O and those of NO$_3^-$ was prepared using US Geological Survey (USGS)-32, USGS-34, USGS-35, and International Atomic Energy Agency (IAEA) NO$_3^-$ standards. Soil NH$_4^+$ in 100-mL extracts of IMT soil was separated onto glass filter papers (GF/D; Whatman) using the diffusion method (69), and then the NH$_4^+$ dissolved on the filter papers was measured for $^{15}$N values on an elemental analyzer coupled with an isotope ratio mass spectrometer (EA-IRMS) (70) at The Ecosystems Center, Marine Biological Laboratory (Woods Hole, MA). IAEA-N$_2$ was run with the samples to check the accuracy of $^{15}$N-NH$_4^+$ data. The analytical precision was better than 0.2‰ for $^{15}$N-NH$_4^+$, 0.5‰ for $^{15}$NO$_3^-$, and 0.5‰ for $^{15}$NH$_4^+$.

Plant N Analyses. Leaf total N concentrations and total $^{15}$N values of plant samples were analyzed using an EA-IRMS (detailed in SI Appendix, Table S1). The analytical precision for $^{15}$N was better than 0.2‰. The leaf NRA assay, which has been used to evaluate the NO$_3^-$-reduction potential of tundra plants by assimilated per either fresh or dry weight (58, 71), was conducted for plants at pristines and control sites of IMT, SAG, TFS-MAT (moist acid tundra), TFS-MNT (moist non-acidic tundra), and at fertilized plots of TFS-MAT (SI Appendix, Table S1 and Fig. S3 A and B). The method of leaf NRA determination was the same as that described in refs. 58, 72, and 73. The NRA data (only those uniformly reported in dry weight) of natural terrestrial plants in low-latitude ecosystems were compiled (SI Appendix, Fig. S3C) for comparing NRA levels between tundra and low-latitude ecosystems.

The concentrations and $^{15}$N and $^{14}$O of NO$_3^-$ in plants were measured using the sensitive denitrifier method (67, 68) at the Tokyo University of Agriculture and Technology (TUAT; method details are described in refs. 74 and 75). In the present study, 1 of 7 root samples of Ericophorum vaginatum and 7 of 94 leaf samples of tundra plants showed measurable NO$_3^-$ concentration as zero, including 5 of 15 Sphagnnum samples, 1 of 8 Cassiope menziesii leaf samples, and 4 of 8 Cassiope menziesii leaf samples. The $^{15}$O values of NO$_3^-$ in plant leaves were determined by combining bacterial reduction [i.e., denitrifier method (67, 68)] and the thermal decomposition method (76). First, NO$_3^-$ in plant extracts was converted to N$_2$O using the denitrifier method (67, 68) at TUAT (method details are described in refs. 74 and 75). Next, the gold-tube conversion of bacteria-produced N$_2$O into N$_2$ and O$_2$ was conducted, and $^{15}$O values (defined as $\Delta^{15}$O = [(1 + $^{15}$O)NaNO$_3$ − 1], where the constant $\beta$ is 0.5247; see refs. 76 and 77) were measured on a Finnigan Delta Plus Advantage IRMS (Thermo Fisher Scientific) at the University of Washington (method details are described in ref. 78). A laboratory standard courtesy of Greg Michalski, Purdue University, West Lafayette, IN [NaNO$_3$ with $\Delta^{15}$O = 19.9‰ (79)] and several standards that mimic the 5% and 10% of atmospheric N$_2$. The $\Delta^{15}$O values for replicate analyses of an individual sample were $\pm 0.2$‰ for $\Delta^{15}$O.

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